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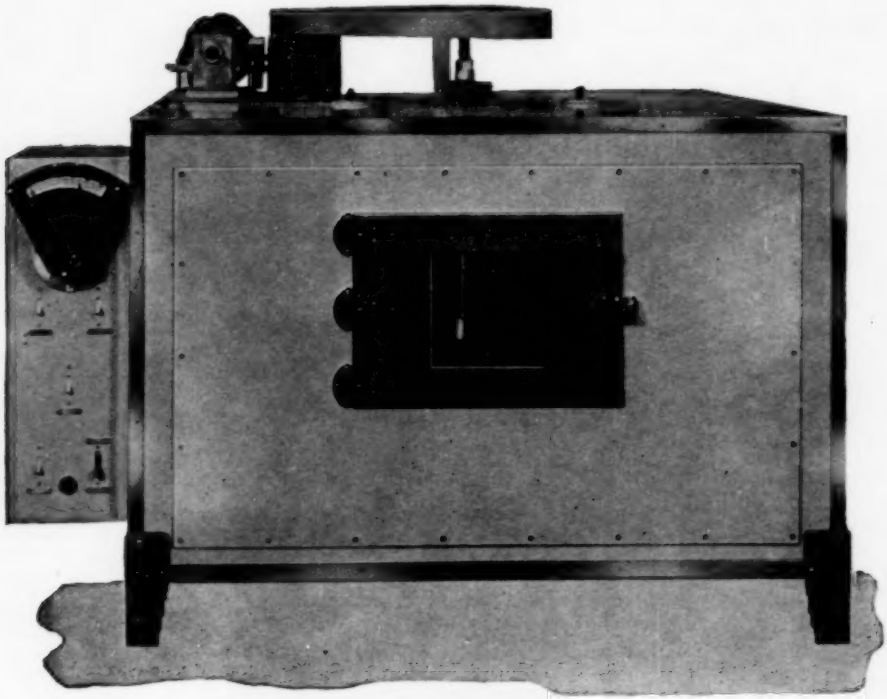

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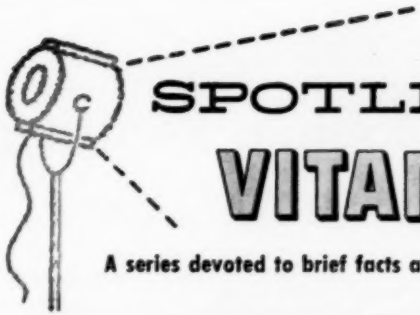

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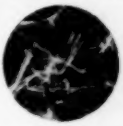

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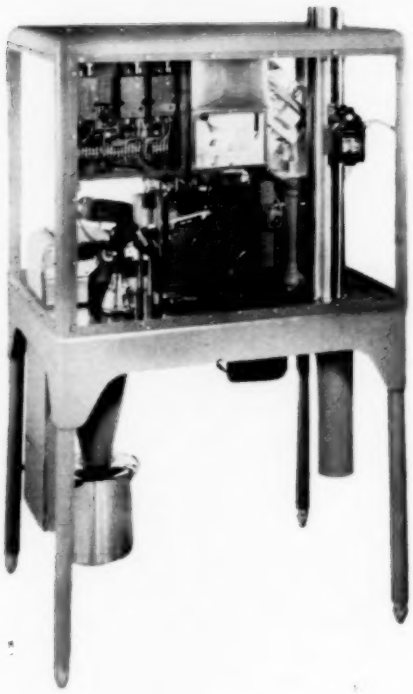

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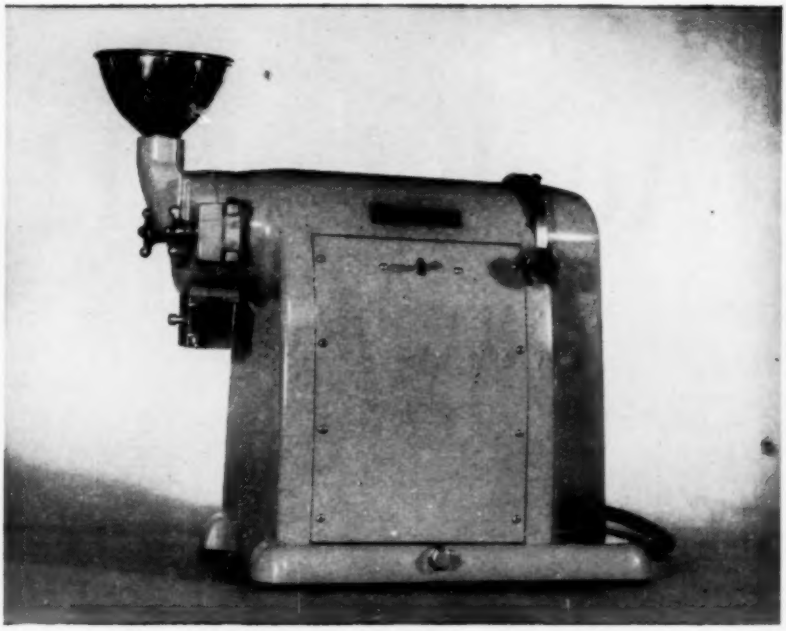

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CEREAL CHEMISTRY

VOL. 35 SEPTEMBER, 1958 NO. 5

# A CALCULATION OF THE SHAPE OF THE ALVEOGRAMS OF MATERIALS SHOWING STRUCTURAL VISCOSITY[1]

A. H. BLOKSMA

**ABSTRACT**

Alveograms have been calculated for materials showing structural viscosity, assuming 1) that the dough bubble remains part of a sphere until rupture occurs, 2) that the volume of the dough bubble increases steadily, 3) that the dough is incompressible, and 4) that every dough element constantly moves perpendicularly to the surface of the bubble at its place. From the resulting graphs it is concluded that, whereas Newton and Maxwell bodies cannot form spherical bubbles in the alveograph, a certain amount of structural viscosity probably favors the spherical shape of the bubble. According to data from the literature, the viscosity of a flour dough decreases faster with increasing rate of strain than is needed to approach the spherical shape most closely. The volumes at which the experimental alveograms attain their maximum heights, together with the sharpness of these maxima, indicate that flour doughs possess a relaxation time as well as structural viscosity.

In a preceding paper (1) an attempt was made to calculate the alveograms of some simple rheological model substances, namely, the (viscous) Newton body, the (viscoelastic) Maxwell body, and the (plastic) Bingham body. It was concluded that Newton and Maxwell bodies cannot form spherical bubbles in the Chopin Alveograph; an assumed spherical shape of the bubble led to the physically absurd result that the pressure in the dough bubble depends considerably upon the position chosen on its surface. In the case of a Bingham body the influence of the chosen position on the calculated pressure is reduced compared with a Newton body, because then the pressure is composed of two terms, that change in opposite directions when moving this position along the surface of the bubble. In the preceding paper (1) the position of an element on the surface was defined by the ratio s/a, which equals zero at the top of the bubble and approaches unity near the base (see definitions in Table I). For a moderate reduction of the influence of s/a upon the pressure P, a considerably greater yield value $p_0$ is needed

[1] Manuscript received July 15, 1957. Contribution from the Institute for Cereals, Flour, and Bread, T.N.O., at Wageningen, The Netherlands.

than for a noticeable contribution of this yield value to the curve height; it has been concluded that in the case of bread doughs the product of the yield value and the fluidity $p_0/\eta$ is too small even for the latter contribution to be noticeable (1).

The visual observation of the expanding dough bubble strongly suggests a spherical shape, although this shape is excluded for Newton, Maxwell, and Bingham bodies because of theoretical considerations and numerical data. It is true that the previous calculations (1) did not ascertain that in the case of these bodies the deviation of the bubble from the spherical shape is sufficiently large to be observed visually. However, the inconsistency between the observation and the theoretical results for the models considered leads one to wonder whether other rheological models are better suited for a description of the behavior of flour dough in the alveograph, that is to say, are more consistent with the apparent spherical shape of the dough bubble. An analysis of the factors that determine the heights of the maxima reveals that, compared with the Newton body, the influence of s/a on the height of the maximum is reduced if the tension is not proportional to the rate of deformation, but increases to a lesser extent with increasing rate of deformation. This implies that the apparent viscosity decreases with increasing rate of deformation; this phenomenon is called structural viscosity. It has been demonstrated with flour doughs by previous investigators (6, 7, 9).

There is a second reason for the use of structural viscosity for a description of the inflation of the dough bubble in the alveograph. In the preceding paper (1), two new measures of the alveogram have been proposed. The first one is the volume $V_m$ of the dough bubble at which the maximum pressure is attained. The second one is the ratio $P_{100}/P_m$, which increases with decreasing sharpness of the maximum (see definitions in Table I). It has been shown that both $V_m$ and $P_{100}/P_m$ increase with increasing relaxation time (cf. Table I of ref. 1); on the other hand, both measures were dependent on s/a. Each of the calculated curves of Fig. 2 of reference 1 gives a combination of $V_m$ and $P_{100}/P_m$; these combinations have been plotted in Fig. 1 (filled circles). The lines connecting points with the same s/a but with different relaxation times are close one to another; the relation between $V_m$ and $P_{100}/P_m$ apparently depends little on s/a. Consequently the relation between $V_m$ and $P_{100}/P_m$ in a series of alveograms of Maxwell bodies with different relaxation times may be expected to approach the bundle of drawn lines in Fig. 1, irrespective of the deviation from the spherical shape that will occur with these materials. Because the drawn lines in Fig. 1 are close to one another

this relation between $V_m$ and $P_{100}/P_m$ can be predicted much more reliably than the shape of the alveogram that corresponds with a given relaxation time. Figure 1 shows that the experimental relationship between $V_m$ and $P_{100}/P_m$, obtained by measurements of some hundreds of alveograms, shows a systematic deviation from the expected behavior of a Maxwell body; the maxima are less sharp than corresponds with the volume at which the maximum curve height is attained. This deviation can be explained, if the doughs show, in addition to a relaxation time, structural viscosity.

For these reasons the author has calculated, in the same way and with identical assumptions as in the preceding paper (1), the alveograms of generalized Newton bodies, showing structural viscosity.

## Calculation

From the many different formulas that have been proposed for a description of structural viscosity, the present author has chosen the equation

$$\eta = \frac{\eta_0 b}{\dot{e}} \cdot \text{arcsinh}\ (\dot{e}/b) \qquad (1),$$

because it has a theoretical basis (3), and because it contains, besides the limiting viscosity, $\eta_0$, only one additional parameter, b. Equation 1

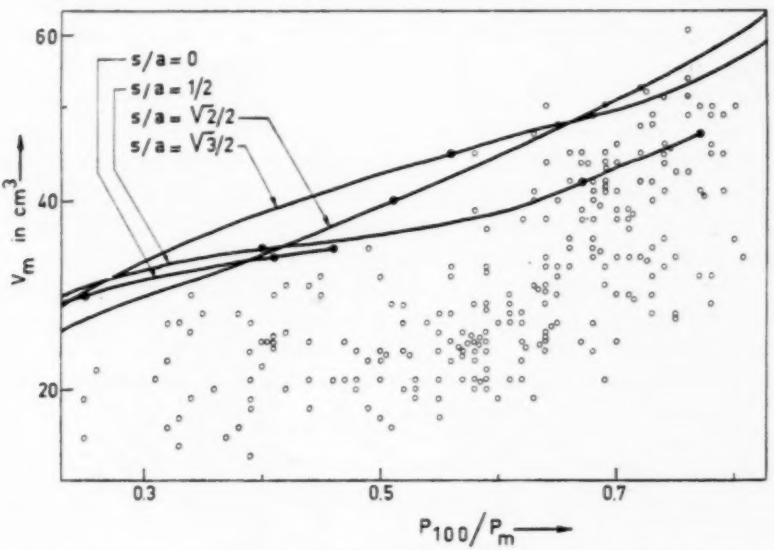


Fig. 1. Relation between $V_m$ and $P_{100}/P_m$. The drawn lines connect points obtained by measurements of the alveograms, calculated for Maxwell bodies with different relaxation times (Fig. 2 of reference 1). The open circles are obtained by measurements of experimental alveograms.

TABLE I

MEANING OF SYMBOLS, AND NUMERICAL VALUES USED IN CALCULATIONS

| SYMBOL | MEANING |
|---|---|
| $a$ | Radius of the base of the dough bubble, 2.762 cm. |
| $s$ | Distance between the position of an element in the dough sheet and the center of the base at the beginning of the experiment. For a given dough element, s, or the dimensionless quantity s/a, remains constant during the inflation; at a given stage of inflation s or s/a determines the position of an element on the bubble surface. |
| $h$ | Height of the dough bubble at an arbitrary time. |
| $\Delta_0$ | Thickness of the original dough sheet, 0.25 cm. |
| $Q$ | Rate of increase of the volume of the dough bubble, 27.0 $cm^3/sec$ |
| $\dot{e}$ | Rate of deformation of a dough element at an arbitrary time and place, namely, half of the relative decrease of the wall thickness per unit time. |
| $\eta$ | Viscosity or, in the case of a Bingham body, the reciprocal of the fluidity. |
| $\eta_0$ | Limiting viscosity for low rates of strain. |
| $b$ | Parameter determining the decrease of the apparent viscosity with increasing rate of strain. At a rate of strain $\dot{e}$ equal to b the apparent viscosity, $\eta$, has decreased to 0.88 of its limiting value. Dimension: $time^{-1}$. |
| $p_0$ | Yield value (for simple tension) of a Bingham body. |
| $P$ | Pressure in the dough bubble at an arbitrary time (and place). |
| $P_m$ | Maximum pressure in the dough bubble (at an arbitrary place). |
| $P_{100}$ | Pressure in the dough bubble at the moment that its volume equals 100 $cm^3$. The ratio $P_{100}/P_m$ is used as a measure of the sharpness of the maximum; in Table I of reference 1 and in reference 2, $P_{100}/P$ has been written instead of $P_{100}/P_m$. |
| $V$ | Volume of the dough bubble at an arbitrary time. |
| $V_m$ | Volume of the dough bubble when P has its maximum value. |

states that, at a low rate of deformation ($\dot{e} \ll b$) the apparent viscosity, $\eta$, approaches $\eta_0$, and that $\eta$ decreases with increasing $\dot{e}$. The values for the parameters $\eta_0$ and b may be chosen in order to adapt equation 1 to the experimental relation between $\eta$ and $\dot{e}$. With appropriate values for these two parameters equation 1 gives a satisfactory description of the published data on bread doughs. According to Eyring's theory (3), b is of the order of the chance that in the unstressed state during 1 second a molecule (or a part of it) jumps from one equilibrium position to an adjacent one.

The calculation proceeds in the same way as in the preceding paper with the exception that the variable $\eta$ from equation 1 is substituted into equation 8 of reference 1. The final result is given in the same way as previously (for the meaning of the symbols see Table I):

$$P = \frac{96\eta_0\Delta_0 Q}{\pi a^4} \cdot F_4 \qquad (2).$$

$$F_4 \equiv \frac{\pi b}{4Q} \cdot \frac{h(a^4 + s^2h^2)^2}{(a^2 + h^2)^3} \cdot \operatorname{arcsinh} \frac{4Q}{\pi b} \cdot \frac{a^2h(a^2 - s^2)}{(a^2 + h^2)^2\,(a^4 + s^2h^2)} \qquad (3).$$

The results of numerical calculations are shown in Fig. 2. The function $F_4$ is plotted against $V/a^3$ for different values of s/a and $Q/ba^3$. Because of the similarity between equation 2 and equations

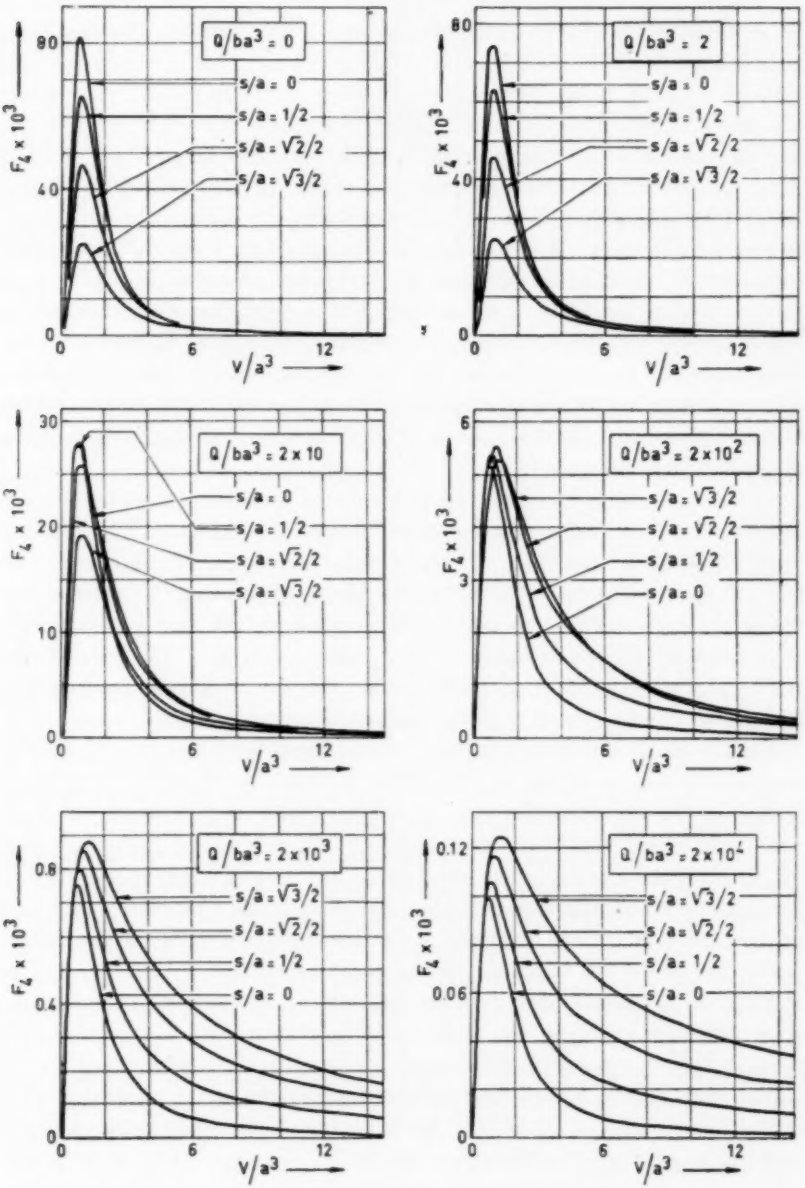


Fig. 2. Theoretical alveograms of materials with structural viscosity. The upper left-hand part refers to a Newton body. A large value of $Q/ba^3$ means that the apparent viscosity decreases rapidly with increasing rate of strain. The value of s/a denotes the position on the surface of the bubble. The curves for $s/a = 1$ coincide with the abscissae in all six parts.

12 and 14 of reference 1, the function $F_4$ can be compared with $F_1$ and $F_2$. For small values of the argument of the arcsinh in equation 3, $F_4$ approaches $F_1$.

## Discussion

Figure 2 shows that, compared with the case of a Newton body ($b = \infty$), the influence of s/a on the shape of the calculated alveograms is reduced if b has a finite value, that is, if the material shows structural viscosity. If for some rheological body the calculated pressure in the bubble depends only slightly upon the chosen position on the surface of the bubble (s/a), then the assumption that the bubble has a spherical shape leads to nearly consistent results. The real bubble formed by such a material in the alveograph will in fact approach a sphere. Therefore it is probable that the spherical shape of the dough bubble in the alveograph is favored by a certain amount of structural viscosity of the dough.

If one considers only the maxima of the calculated curves, the influence of s/a is minimal when $Q/ba^3$ is of the order of $2 \times 10^2$, that is, when b is $7 \times 10^{-3}\,sec^{-1}$. From this value downwards there is a wide range of values of b for which the positions of the maxima of the calculated curves depend only little upon s/a. However, one should not conclude from the coincidence of the maxima of the four drawn curves that the position of the maximum is completely independent of s/a; the curve for $s/a = 1$ still coincides with the abscissa.

In the case of a Newton body, curve heights of the same order of magnitude are calculated for different parts of the dough bubble. This is caused by the fact that the top of the bubble (s/a small) shows a high rate of deformation but has a thin wall, whereas the lower part of the bubble (s/a approaching unity) is deformed more slowly but has a greater wall thickness. With decreasing b the influence of the rate of deformation upon the curve height decreases. Consequently the curves for high s/a tend to rise in respect of those for low s/a. This tendency manifests itself first in the tails of the curves, because in a later stage of inflation the differences between the rates of deformation at different parts of the bubble are smaller than at the beginning; in the tails it can already be observed when the maxima nearly coincide ($Q/ba^3 = 2 \times 10^2$). At still lower values of b also the height of the maximum of the curve increases when s/a increases from 0 to $\sqrt{3}/2$. One can obtain a qualitatively similar set of curves by a linear combination of the functions $F_1$ and $F_3$ (see Figs. 2, upper left, and 4 of ref. 1); this can be understood readily because the behavior of a Bingham body is a special case of structural viscosity.

Does the value of b that is needed for a reasonable coincidence of the curves for different s/a agree with data from the literature about the dependence of the viscosity upon the rate of deformation? An imperfection of equation 1 hinders answering this question. Equation 1 links up b with the particular choice of the coordinate system and with a definition of $\dot{e}$ that does not cover other types of deformation as, for instance, simple shear. Because the viscosity, $\eta$, is a scalar it seems logical that it is not a function of a tensor component but rather of another scalar; a plausible scalar is the rate of dissipation of energy caused by the viscous flow (8). The assumption that the rate of dissipation of energy completely determines the viscosity permits a comparison between data from different types of deformation. Then data from Schofield and Scott Blair (9) and from Halton (7)[2] correspond with $b = 3 \times 10^{-4}\,sec^{-1}$, and data from Halton and Scott Blair (6) with $b = 1 \times 10^{-4}\,sec^{-1}$. According to these values the viscosity decreases considerably faster with increasing rate of strain than is needed for a maximum reduction of the influence of s/a upon the course of the curves. The sets of curves that correspond with these values of b are approached by the two lower parts of Fig. 2; although the coincidence of the curves is not maximal, the influence of s/a upon their course is less than for Newton or Maxwell bodies. Further, one should realize that a comparison of the process in the alveograph bubble with the experiments of Schofield, Scott Blair, and Halton involves a far extrapolation; in these experiments the rate of extension did not exceed $5 \times 10^{-3}\,sec^{-1}$, whereas in the alveograph bubble $-\dot{e}_R$ attains a maximum value of $5.3 \times 10^{-1}\,sec^{-1}$.

Figure 2 shows that the sharpness of the maximum of the alveogram decreases as a consequence of structural viscosity; the volume $V_m$ at which this maximum is attained, however, hardly increases. Therefore structural viscosity may explain why the experimental relation between the sharpness of the maximum (measured by $P_{100}/P_m$), and the volume at which this maximum is attained ($V_m$), does not correspond with the relation to be expected for Maxwell bodies (compare Fig. 1). That $V_m$ is often considerably greater than 20 $cm^3$ cannot be explained by structural viscosity; on the other hand, it is explained by a relaxation time (1). Therefore, with our present insight into the inflation process in the alveograph, we may consider the shape of the experimental alveograms as an indication that flour doughs possess a relaxation time as well as structural viscosity. A quantitative treatment of the joint effect of these properties will not be attempted here.

[2] It has been assumed that these data have been obtained from the same type of experiments as the data in references 6 and 9.

A flat maximum of the alveogram, if it is coupled with a high $V_m$, may be an indication of a long relaxation time. On the other hand, if $V_m$ is low, a flat maximum indicates considerable structural viscosity. Of the two measures, $V_m$ and $P_{100}/P_m$, that have been proposed previously (1), the first one cannot be measured with sufficient accuracy; therefore only $P_{100}/P_m$ remained for practical use. The result of the present study is, however, that a high $P_{100}/P_m$ may be an indication either of a desirable (4, 6) long relaxation time or of a harmful (5, 6) structural viscosity. This might explain why the present author failed to find a high correlation between $P_{100}/P_m$ and the loaf volume (2).

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# EFFECT OF DRYING ON THE PRESENCE OF CAVITIES IN CORN STARCH GRANULES[1]

ROY L. WHISTLER, W. W. SPENCER, J. L. GOATLEY, AND Z. NIKUNI[2]


## ABSTRACT

The way different drying conditions affect the production of cavities in corn starch granules has been examined by observation of granule sections in an electron microscope. Starch granules were dried in air at room temperature (about 27°C.), at 45°C., and at 60°C. As the moisture content of the granules decreased the number possessing cavities increased, but the number of cavitated granules at any particular moisture content above 20% also increased with the temperature of drying. Starch granules dried at room temperature to moisture contents below 20% had fewer cavities than granules dried at 45°C. and 60°C. where the number of cavitated granules was equivalent.

Starch from the floury endosperm of field-matured kernels (moisture content 14%) gave 23% of the sections with cavities; starch from horny endosperm showed 27%. Floury endosperm starch from undried kernels of 50% moisture content had cracks in 13% of the sections; horny endosperm starch from the same kernels had 25% of the sections with cracks.

Serially cut sections showed that cavities of all granules broach the surface at some point. Additional evidence for surface opening of cavities was that cavitated granules, when soaked in mercuric chloride solution, showed salt penetration from the walls of the cavity as well as from the surface of the granule.


Cavities or fissures in starch granules have been observed by many workers and were reported as early as 1913 by Reichert (4). Observations were made with both electron (7, 9, 10) and light microscopes, but no satisfactory explanation has been given for the origin of the cavities. Whistler and Thornburg (9) reported that cavities or fissures are rarely observed in undried granules but are commonly found in mature dried granules.

This paper presents additional information on the nature of these cavities and on how the number of cavitated granules can be controlled in part by proper selection of drying temperature.

## Materials and Methods

*Isolation of Starch.* Undried corn kernels of approximately 50 to 60% moisture content were obtained from common yellow field corn. The corn kernels were ground in water in a Waring Blendor and filtered through fine bolting cloth; the filtrate was centrifuged, and the protein layer scraped from the starch as much as possible. Subse-

[1] Manuscript received August 15, 1957. Journal Paper No. 1146 of the Purdue University Agricultural Experiment Station. Contribution from the Department of Biochemistry, Purdue University, West Lafayette, Indiana.

[2] Present address: Institute of Scientific and Industrial Research, Osaka University, Sakai, Osaka, Japan.

quently, a portion of the starch was extracted with 0.06*N* sodium hydroxide solution for 2 hours to further remove protein, and then was neutralized with 0.1*N* hydrochloric acid solution.

*Drying and Preparation of Samples.* Samples of corn starch were dried at room temperature (about 27°C.), 45°C., and 60°C. to the desired moisture content. Drying at 45° and 60°C. was performed in an air oven. After drying at one of the above temperatures, a portion of the sample was removed and placed in 0.05*M* mercuric chloride solution; a second portion was placed in dioxane; and a moisture determination was made on an additional portion. Moisture was determined by drying to a constant weight at 110°C. in an air oven. Samples placed in mercuric chloride solution were allowed to soak for 6 hours, filtered on fritted glass, and washed with and placed in dioxane.

Horny and floury endosperm were isolated both from mature, dried kernels and from undried kernels (6 weeks after pollination) of 50% moisture content by dissecting the kernel and removing the desired portions. The starch was isolated from each as described above and was not treated with base or acid. A portion of each sample of starch was placed in mercuric chloride solution for 6 hours.

Samples were embedded in methacrylate and sectioned for electron microscopy as described by Whistler and Thornburg (9).

*Serial Sectioning.* Serial sections of starch granules were made using a wedge-modified Spencer Model 820 rotary microtome with a rocking glass knife. Sections were made by the method of Gay and Anderson (2). All serial sections were made from endosperm of single-cross, common yellow field corn. Kernels were killed, fixed (6), dehydrated, and embedded (9). The methacrylate block was trimmed under the dissecting microscope to leave a surface rectangle of about 0.08 by 0.06 mm. Sections of 0.2-$\mu$ thickness were made and mounted on collodion-coated slot grids.[3]

*Examination for Birefringence.* A small slice of each methacrylate block was removed with a razor blade, except for those blocks prepared for serial sectioning, and the slice placed under a cover slip on a microscope slide. The methacrylate was dissolved with toluene. The granules were then examined for birefringence using a polarizing light microscope.

*Examination of Samples in the Electron Microscope.* All sections from samples described above were leached for 30 seconds with toluene to remove the methacrylate embedding material. The serial sections of corn endosperm were shadowed with chromium at an 18° angle.

[3] Grids were obtained from Ernst F. Fullam, Inc., P.O. Box 444, Schenectady, New York.

Similar shadowing was applied to all sections shown in this paper to bring out greater detail. However, other sections examined were not shadowed, so that penetration by the mercuric chloride solution might be more accurately observed. The electron microscope was the RCA EMU-2D.[4] Cracks in sections were counted at 1300 × magnification.

The percentage of granule sections with cracks or fissures was determined by counting three separate groups of 100 sections each and taking the average value of these counts. In no case did the three 100-section counts vary by more than four. Only granule sections greater than 8$\mu$ in diameter were counted. Counted sections would thus include sections from granules covering most of the size range (usually given as 4 to 26$\mu$ (8)). Very small granules and tiny sections cut from near the periphery of the granule would be excluded.

## Results

In all serial sections where cavities were seen, the cavities extended to the surface of the granule in at least one of the sections, as illustrated in Fig. 1 (A and B, granule m). The cavities shown in both Fig. 1 and Fig. 2 are chosen to depict the lacelike network sometimes seen in granules of endosperm sections. On examination of granules soaked in mercuric chloride solution, over 96% of those containing cavities showed penetration of the salt from the cavity-walls as well as from the exterior of the granule. These diffusion data indicate also that most, if not all, cavities in granules extend to the surface of the granule and that few, if any, additional cavities are formed during the embedding and sectioning processes. There was no significant difference between the percentage of cavities occurring in mercuric chloride-treated starch and untreated starch dried to the same moisture content. Pretreatment of starch with base and acid had no effect on the appearance of cracks. All samples retained their birefringence after embedding. Starch granules treated in a Waring Blendor showed no increase in cavitation.

A comparison of the number of cracks and fissures produced under different drying conditions is given in Table I. The percentage of cavitated granules increased markedly as the moisture content of the granules decreased. In addition, the percentage of cavitated granules at any moisture level increased with the temperature at which the granules were dried, except when granules were dried to below 20% moisture. Granules dried to below 20% moisture at 45° and 60°C. showed the same number of cavities.

Starch dried in a stream of air at 60°C. to a moisture content of 12.5% contained cracks in 35% of the sections examined. On the other

[4] Radio Corporation of America, Engineering Products Division, Camden, N. J.

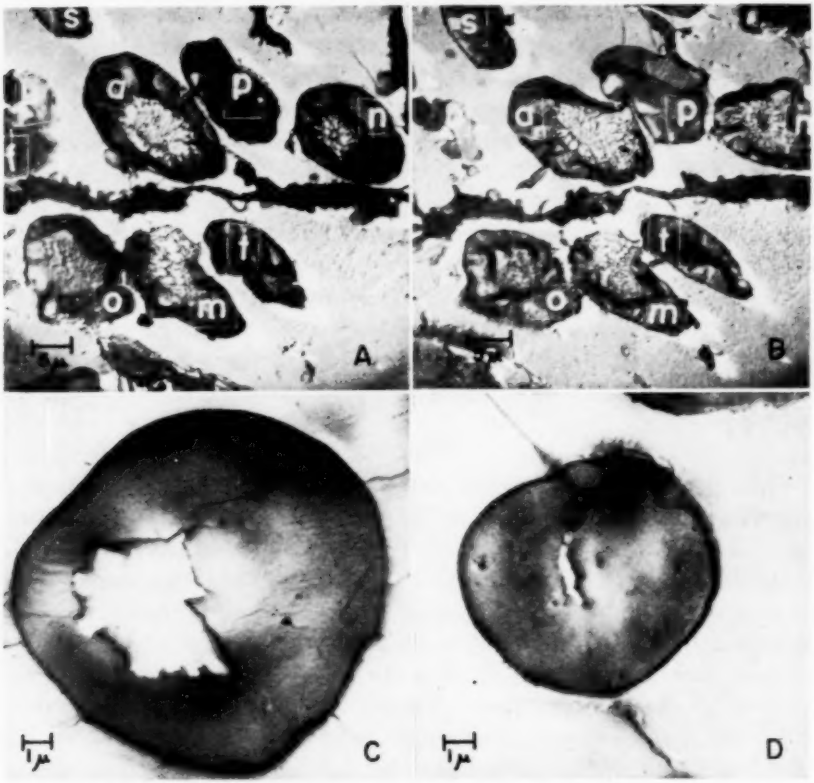


Fig. 1. Chromium-shadowed sections of corn starch granules. A and B, two serial sections of endosperm; letters indicate sections of the same granule. C and D, isolated, dried starch granule showing common form of cavities.

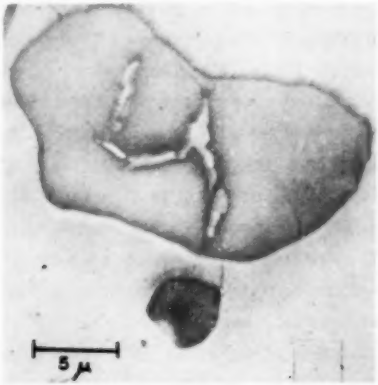


Fig. 2. Chromium-shadowed section of isolated, dried corn starch granule, showing extensive cavitation which is occasionally found.

TABLE I

EFFECT OF DRYING TEMPERATURE AND MOISTURE CONTENT ON THE NUMBER OF CRACKED STARCH GRANULE SECTIONS

| Moisture of Dried Starch | Sections with Cracks | | |
|---|---|---|---|
| | Dried at Room Temperature (About 27°C.) | Dried at 45°C. | Dried at 60°C. |
| % | % | % | % |
| 45.5 | 15 | ... | ... |
| 43 | ... | ... | 34 |
| 38 | ... | 26 | 41 |
| 33.5 | 16 | ... | ... |
| 31 | ... | 27 | 49 |
| 30 | 16 | ... | ... |
| 29 | ... | ... | 49 |
| 26 | ... | 37 | ... |
| 23.5 | 22 | ... | ... |
| 19 | 24 | ... | ... |
| 15.5 | ... | 48 | ... |
| 14.5 | ... | 50 | ... |
| 13.5 | 28 | ... | 52 |
| 12 | ... | 51 | ... |
| 6.4 | ... | ... | 54 |

hand, a sample of starch of 40% moisture content, when dried to constant weight in an air oven at 110°C., showed cracks in 55% of the sections examined.

As the moisture content of the corn kernel was lowered there was a marked increase in the number of cracked sections in the floury endosperm starch, while sections of starch from the horny endosperm showed a relatively small increase, as shown in the table below.

| *Moisture of Kernel* | *Cracked Horny Endosperm Sections* | *Cracked Floury Endosperm Sections* |
|---|---|---|
| % | % | % |
| 50 | 25 | 13 |
| 14 | 27 | 23 |

## Discussion

The percentage of cavitated starch granules must exceed the percentage of sections containing cracks. This is evident because it is possible to cut a section from a granule which contains a cavity without slicing through the cavity. The percent of cavitated granules is difficult to estimate, because the size and shape of the cavities have such wide variations.

Since birefringence is still present in the granules after all the treatments, it is apparent that cavitation does not greatly affect molecular orientation within the granule.

Commercially produced starches vary considerably in physical properties, and for a number of starches these differences have some

correlation with the time and temperature of drying (1, 3, 5). Physical properties, such as pasting characteristics and viscosity, can quite conceivably be related to the extent of granule cavitation. In addition, the increase in granule surface area due to cavitation likely would affect chemical reactivity. Examination of the extent of cavitation produced by various industrial drying methods may aid the starch industry in controlling the properties of its products.

**Acknowledgment**

The authors wish to express their thanks to the Corn Industries Research Foundation for their kind support of this work.

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# THE CONVERSION OF $Br^{82}$-LABELED BROMATE TO BROMIDE IN NONFERMENTING AND FERMENTING DOUGHS AND IN BAKED BREAD[1]

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## ABSTRACT

Potassium bromate labeled with $Br^{82}$ was prepared by electrolytic oxidation of potassium bromide-$Br^{82}$. Conversions of the labeled bromate to bromide were investigated for initial bromate concentrations of 5, 10, 15, 20, and 30 p.p.m. in nonfermenting and fermenting doughs and baked bread prepared from an experimentally milled flour. Reduction of part of the bromate to bromide was found in all cases, the rates of their conversion ranging from 0.08 to 0.36 p.p.m. per hour for the nonfermenting doughs and 0.25 to 1.05 p.p.m. per hour for the fermenting doughs. The amounts of bromate converted to bromide were greater in the bread than in the corresponding fermenting doughs when these doughs were ready for baking, indicating reductions of further quantities of the bromate during baking. While these observations do not constitute proof of any mechanism of improver action, the findings did show unequivocally that some oxidation-reduction reactions have taken place and would, therefore, be consistent with mechanisms involving oxidation-reduction reactions.


As one of the lines of attack that may aid in elucidating the nature of the improver effect of bromate, Anderson and his associates (5, 6) have recently studied the disappearance of bromate in fermenting and nonfermenting doughs by amperometric titration. The measurement, by ordinary analytical means, of minute amounts of improver in relatively large quantities of flour, salt, sugar, and other dough constituents is, at best, rather difficult. Thus Cunningham and Anderson (5) were able to follow the rates of decomposition of bromate only for initial bromate concentrations of 15 and 30 p.p.m. The application of radioactive tracers, with its high sensitivity and the ease of identification of the labeled compounds by the carrier technique, provides an excellent tool for determining the fate of flour improvers in dough systems as well as in the baked bread. Conn and co-workers (4), for example, have used $I^{131}$-labeled potassium iodate to investigate the decomposition of iodate during the baking of bread, and have shown the major decomposition product to be iodide. The present paper reports observations on the conversion of $Br^{82}$-labeled potassium bromate to bromide in fermenting and nonfermenting doughs and in baked bread.

[1] Manuscript received October 7, 1957. Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Sask. Issued as paper No. 343 of the Associate Committee on Grain Research, National Research Council of Canada.

## Materials and Methods

*Potassium Bromate-$Br^{82}$.* Bromine-82, half-life 35.9 hours (3), was obtained from Atomic Energy of Canada Limited, Chalk River, Ontario, through service irradiation of potassium bromide. After 5 days of irradiation with a neutron flux of $7 \times 10^{12}$ n/cm$^2$/sec, the specific activity of the potassium bromide, on leaving the pile, was approximately 1.3 mc/mg. Because of its relatively short half-life, the effective working time of this material was only about 2 weeks. Throughout this 2-week period, the radiochemical purity of the bromine-82 was ascertained by following its decay pattern and frequently calculating its half-life.

The radioactive potassium bromide was converted to potassium bromate by electrolytic oxidation (7, 8). The electrolytic cell was constructed from a piece of 8-mm. i.d. Pyrex glass tubing sealed at one end. The electrodes were made of smooth platinum foil, with anode area of 1 cm.$^2$ and cathode area of 0.5 cm.$^2$ These electrodes were bent into semicylindrical shape so as to fit into the 8-mm. tubing and were mounted close together (approximately 2 mm. apart) to ensure high current density before the lead wires were sealed through the wall of the cell.

In a typical oxidation, with all operations carried out behind a lead shield, a solution of 250 mg. of potassium bromide-$Br^{82}$ (containing 50–100 mc. of radioactivity) in 0.40 ml. of distilled water and 0.40 ml. of 0.1*N* hydrobromic acid containing 2.0 mg. of potassium dichromate as a depolarizer was placed in the cell. Electrolysis was started with a current of 60 milliamperes and a potential of 2.3 volts. The current was maintained at 50–60 milliamperes for 4–5 hours. As electrolysis was proceeding, crystals of potassium bromate were deposited. After 250 milliampere-hours had passed through the cell, the electrolysis was stopped and the cell heated over a steam bath to effect dissolution of the potassium bromate-$Br^{82}$. The solution was pipetted into a small centrifuge tube in which the labeled potassium bromate crystallized after the solution was cooled in an ice bath. The yield of potassium bromate obtained in this way generally ranged between 60 and 65%. The product was recrystallized twice more from water before being used for improver studies. A solution of this labeled bromate was shown to be free from bromide, as the silver bromide obtained from fractional precipitation after addition of appropriate amounts of carriers was not radioactive.

*Conversion of Bromate to Bromide in Nonfermenting and Fermenting Doughs and in Baked Bread.* A straight flour with good bromate re-

sponse, experimentally milled from a sample of Thatcher wheat with high protein content, was used in all experiments reported in this paper. The milling was carried out in a Buhler mill to 72% extraction. The resulting flour contained 17.0% protein (N × 5.7) and 0.56% ash on a 14.0% moisture basis.

Nonfermenting doughs were formed from 200 g. of flour containing 5, 10, 15, 20, or 30 p.p.m. labeled potassium bromate and 127 ml. of distilled water. For each bromate level, 20-g. subsamples of the dough were removed at different time intervals and extracted, and the amount of bromate and bromide recovered in the extract determined as described in subsequent sections of this paper.

All fermenting doughs were prepared using the following baking formula: 100 g. flour, 3 g. yeast, 5 g. sucrose, 1.75 g. sodium chloride, 3 g. shortening, 4 g. nonfat dry milk, 0.1 g. ammonium dihydrogen phosphate, 0.3 g. nondiastatic malt, labeled potassium bromate (5, 10, 15, 20, or 30 p.p.m.), and absorption to suit (63.5%). For each bromate level, four batches of such doughs were prepared and allowed to ferment and worked according to the usual baking test procedure. The four batches were analyzed by extraction of duplicate 20-g. subsamples and measurements of bromate and bromide contents in the extracts immediately after mixing, first punch, second punch, and proofing.

Duplicate loaves of bread were baked using the baking formula listed above. Assays for labeled bromate and bromide were made on extracts obtained from a 40-g. subsample of each loaf.

*Extraction.* Aqueous extracts of subsamples of dough or bread were obtained by a method similar to that used by Cunningham and Anderson (5). A 20-g. subsample of dough was dispersed in 50 ml. of water in a Waring Blendor operated at high speed for 2 minutes. A few drops of n-octyl alcohol were added as antifoaming agent where necessary. To aid the removal of colloidal materials, solutions of zinc sulfate (155 g. per liter) and sodium hydroxide (40 g. per liter) were introduced. As it is known that there is no exchange between radioactive bromide and bromate ions in a neutral aqueous medium (12), to each dough dispersion 15 ml. of zinc sulfate solution were first added. This was followed by titration with the sodium hydroxide solution to a pH of 7, using phenol red as indicator. After centrifuging at 2000 r.p.m. for 3 minutes, crystal-clear aqueous extracts were obtained. The total volume of extract, used in subsequent calculations, was taken as the sum of all water introduced, including the amount derived from the subsample of dough based on the 63.5% absorption.

Extraction of baked bread was carried out in a similar way except that a 40-g. subsample was dispersed in 150 ml. of water, followed by

the addition of 30 ml. of zinc sulfate solution and then neutralization with 1*N* sodium hydroxide.

*Measurements of Radioactive Bromate and Bromide.* All radioactivity measurements were made in a windowless gas flow Geiger counter with scaler. Aliquots of liquid samples, 0.5- or 1.0-ml., were evaporated to dryness on aluminum pans[2] for the counting. To eliminate creeping along pan edges during evaporation and to aid in formation of dried sample of uniform thickness and geometry, a circle was drawn with grease pencil near the edge of each pan used; the liquid being evaporated was thus confined within the marked circle.

Total recovery of $Br^{82}$-labeled compounds from each experimental sample of dough or bread was measured by drying and counting an aliquot of the aqueous extract. Immediately before and after the counting of each experimental sample, a sample containing a known amount of $Br^{82}$-labeled potassium bromate, to be designated as the standard, was also counted. By this "sandwiching" technique, the radioactivity in each experimental sample was related quantitatively to the original bromate dosage by comparison with the average value of the two counts for the standard. No decay correction is necessary in the calculation since the $Br^{82}$ isotope in both the experimental sample and the standard would have decayed to the same extent at the time of counting. As a further precaution against possible variations in sample thickness and geometry between the standard and experimental samples, the standard solution, from which an aliquot was evaporated to dryness to give the standard, was prepared by adding a known quantity of labeled potassium bromate to an aqueous extract from nonradioactive dough or bread obtained in the same manner as in the extraction of the subsamples of dough or bread containing labeled bromate.

As an illustration of actual experimental data, take, for example, the fermenting dough containing 5 p.p.m. labeled potassium bromate which was worked up after the first punch.

*Standard solution:* 0.01 mg. of $KBr^{82}O_3$ in 14.52 ml. of extract from a subsample of nonradioactive fermenting dough; 0.5 ml. of the standard solution was evaporated to dryness to give the standard.

Therefore, weight of $KBr^{82}O_3$ in standard $= 3.44 \times 10^{-4}$ mg.

*Experimental sample:* Total weight of dough = 177 g.

Weight of dough extracted = 21.3 g.

Total volume of extract = 85.9 ml.

Aliquot of extract counted = 0.5 ml.

Activity of experimental sample = 1918 counts/minute

Activity of standard (average of two determinations made immediately

[2] Sample pans, 1¼ in. in diameter, supplied by Nuclear-Chicago Corp.

before and after the counting of the experimental sample) = 2003 counts/minute

Therefore, labeled compounds in aliquot of extract counted, expressed as weight of $KBr^{82}O_3 = (1918/2003) \times 3.44 \times 10^{-4}$ mg.

Hence, total recovery of extracted labeled compounds, expressed as weight of $KBr^{82}O_3 = (1918/2003) \times 3.44 \times 10^{-4} \times (85.9/0.5) \times (177/21.3) = 0.47$ mg.

In a duplicate run, total recovery was equivalent to 0.49 mg. of $KBr^{82}O_3$.

Therefore, the averaged total recovery was equivalent to 0.48 mg. of $KBr^{82}O_3$.

Original $KBr^{82}O_3$ dosage = 5 p.p.m., i.e., 100 g. of flour and 0.50 mg. of $KBr^{82}O_3$.

Thus, total recovery in aqueous extract = $(0.48/0.50) \times 100 = 96\%$.

For the determination of the amount of bromate converted to bromide, a method, similar to that used by Libby (12), involving the fractional precipitation of silver bromide, was adopted. To a 10-ml. aliquot of the aqueous extract of dough or bread was added 1.0 ml. of solution containing 30.00 mg. of potassium bromide and 30.00 mg. of potassium bromate as carriers. After thorough mixing, 1.0 ml. of solution containing 42.82 mg. of silver nitrate (the molar equivalent of 30.00 mg. of potassium bromide) was introduced. The mixture was well shaken and the silver bromide centrifuged down.[3] An aliquot of the supernatant was evaporated to dryness and its radioactivity measured. The standard was also used to "sandwich" the sample in the counting as described under determination of total recovery of labeled compounds.

For an illustration of actual experimental data, the fermenting dough containing 5 p.p.m. labeled potassium bromate which was worked up after the first punch will again be taken as an example. As before:

Weight of $KBr^{82}O_3$ in standard = $3.44 \times 10^{-4}$ mg.

Total weight of dough = 177 g.

Weight of dough extracted = 21.3 g.

Total volume of extract = 85.9 ml.

Ten milliliters of the extract were treated with 1.0 ml. of solution of carriers and 1.0 ml. of silver nitrate solution, giving rise to 12.0 ml. of supernatant solution after the removal of silver bromide.

Five-tenths milliliter of the supernatant was evaporated to dryness and counted.

[3] In some experiments, an added precaution was made to ensure no precipitation of silver bromate by heating the mixture containing the silver bromide over a steam bath before centrifugation. However, this was later found to be unnecessary.

Activity of 0.5 ml. aliquot of supernatant solution = 1266 counts/minute.

Activity of standard (average of two determinations made immediately before and after the counting of the experimental sample) = 1987 counts/minute.

Therefore, labeled compounds in the aliquot of supernatant counted, expressed as weight of $KBr^{82}O_3 = (1266/1987) \times 3.44 \times 10^{-4}$ mg.

Hence, total extracted labeled compounds, with all bromide ions removed, expressed as weight of $KBr^{82}O_3 = (1266/1987) \times 3.44 \times 10^{-4} \times (12.0/0.5) \times (85.9/10.0) \times (177/21.3) = 0.38$ mg.

In a duplicate run, the total extracted labeled compounds after bromide removal was found to be equivalent to 0.40 mg. of $KBr^{82}O_3$. Therefore the average for the two runs gave 0.39 mg. $KBr^{82}O_3$ remaining after removal of all bromide ions.

Since the total recovery of extracted labeled compounds expressed as weight of $KBr^{82}O_3$ was 0.48 mg., the amount of $KBr^{82}O_3$ converted to bromide was 0.48–0.39 = 0.09 mg. *or* $(0.09/0.50) \times 100 = 18\%$ of the 5-p.p.m. dosage.

To confirm the reliability of this method of estimating the amount of bromate converted to bromide, occasional samples of dough were also analyzed by an entirely different procedure. This involved the removal of bromide ions through exchange with bromine, the latter in turn being separated by extraction with an organic solvent (2). To a 10-ml. aliquot of the aqueous extract of dough, 30 mg. of potassium bromate were added as hold-back carrier. Approximately 100 mg. of bromine were next introduced. After thorough shaking, the mixture was extracted with carbon tetrachloride until all bromine coloration was removed. Since bromide ions would exchange rapidly with bromine, all $Br^{82}$ present as bromide would have been removed with the bromine. After extraction of bromine, an aliquot of the aqueous solution was evaporated to dryness and counted in conjunction with the standard, giving radioactivity data from which one could calculate the weight of $KBr^{82}O_3$ remaining after the removal of bromide and hence the amount of bromate converted to bromide. Very good agreements were obtained for the samples analyzed by both the fractional precipitation of silver bromide and the procedure involving the exchange of bromide with bromine, indicating that either one of these methods would be suitable for estimating the degree of conversion of labeled bromate to bromide. These agreements also further indicate that there was no exchange between bromate and bromide ions in these experiments, for, if there were, the two methods used for the removal of bromide would be unlikely to give rise to the same results.

## Results and Discussion

The total recoveries of $Br^{82}$-labeled compounds in the aqueous extracts of nonfermenting or fermenting doughs were generally in the order of 90%. There was no apparent relation between the degree of total recovery and any experimental variables such as initial bromate concentration or time of reaction. For example, in the fermenting dough containing 5 p.p.m. of bromate, total recoveries were 90%, 96%, 94%, and 90%, respectively, for the samples analyzed after mixing, first punch, second punch, and proofing. Similarly, for the nonfermenting dough containing 15 p.p.m. of bromate, total recoveries were 96%, 91%, 89%, 92%, and 92%, respectively, for subsamples worked up at 20, 80, 145, 200, and 265 minutes after the beginning of mixing. For all the nonfermenting dough samples analyzed, the average value for total recovery of $Br^{82}$ activity in the aqueous extracts was 92%, while for all the fermenting doughs, the average value was 91%. Hence, on the average, nearly 10% of the original dose of potassium bromate could not be extracted from the doughs under the experimental conditions which were used. No attempts were made to elucidate the chemical nature of this unextractable fraction of $Br^{82}$ activity. Possibly, some of the $Br^{82}$-containing ions were adsorbed tenaciously by other dough components, or some of the bromate might have been changed into water-insoluble forms through interaction with other dough constituents, or both.

Of the $Br^{82}$ activity in the aqueous dough extracts, the amounts present as bromide, expressed as a percentage of the initial bromate converted to bromide, are plotted against time after the beginning of mixing in Fig. 1. Graphs A, B, C, D, and E represent initial bromate levels of 5, 10, 15, 20, and 30 p.p.m., respectively. From the slopes of these graphs, the rates of conversion of bromate to bromide were calculated and these are given in Table I.

It is of interest to compare the present results with the rates of bro-

TABLE I

RATE OF CONVERSION OF BROMATE TO BROMIDE IN NONFERMENTING AND FERMENTING DOUGHS

| Initial Bromate (IB) Level | Rate of Conversion | | | |
|---|---|---|---|---|
| | Nonfermenting | | Fermenting | |
| *ppm* | *% IB/hour* | *ppm/hour* | *% IB/hour* | *ppm/hour* |
| 5 | 1.5 | 0.08 | 5 | 0.25 |
| 10 | 2.5 | 0.25 | 7 | 0.70 |
| 15 | 1.5 | 0.23 | 5.4 | 0.81 |
| 20 | 1.2 | 0.24 | 4.5 | 0.90 |
| 30 | 1.2 | 0.36 | 3.5 | 1.05 |

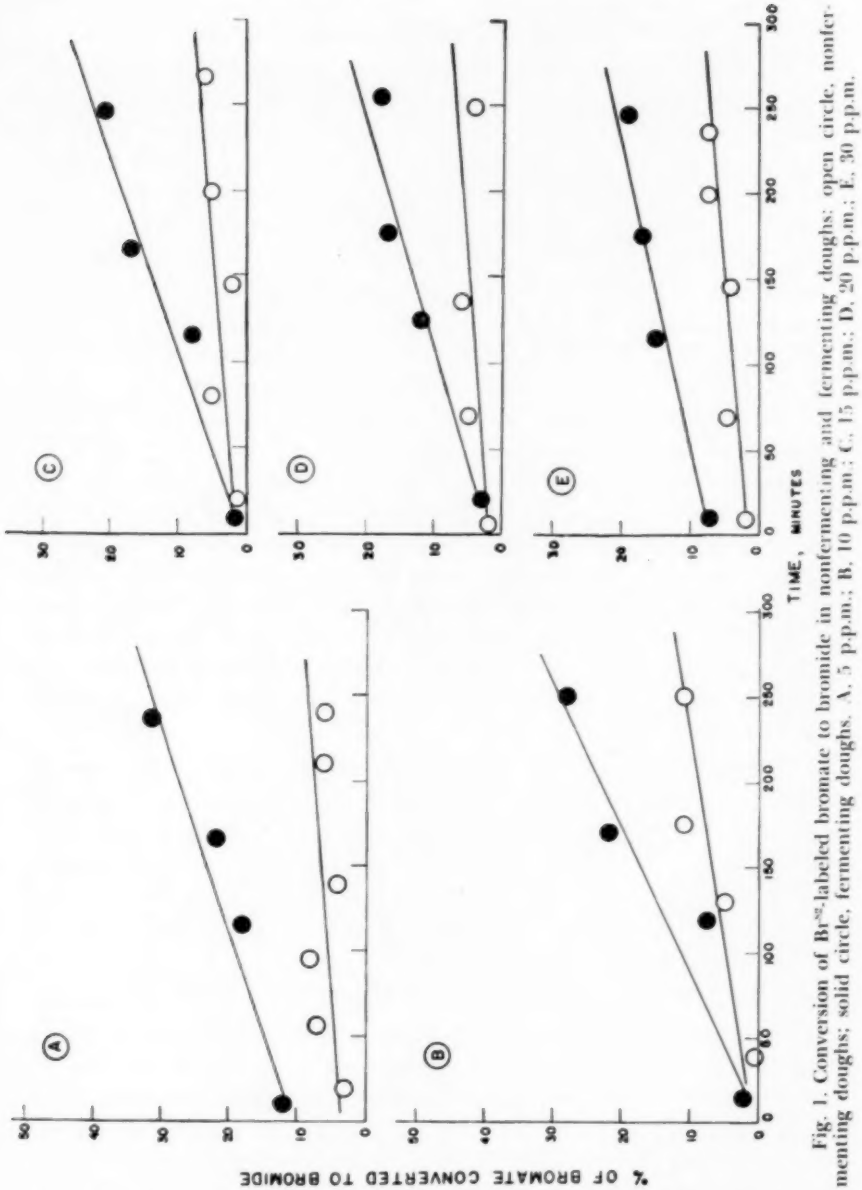


Fig. 1. Conversion of $Br^{82}$-labeled bromate to bromide in nonfermenting and fermenting doughs; open circle, nonfermenting doughs; solid circle, fermenting doughs. A, 5 p.p.m.; B, 10 p.p.m.; C, 15 p.p.m.; D, 20 p.p.m.; E, 30 p.p.m.

mate loss reported by Cunningham and Anderson (5). These workers found that "in both fermenting and nonfermenting doughs mixed in nitrogen, a small initial loss of bromate is followed by a continuing loss at uniform rate." In our aqueous dough extracts, there was no sharp

initially large conversion of bromate to bromide for the samples analyzed immediately after mixing. Possibly, the initial loss of bromate recorded by Cunningham and Anderson might be due to that fraction of bromate which could not be extracted by water from the doughs. For the continuing loss of bromate after the initial loss, the rates reported for nonfermenting and fermenting doughs mixed in nitrogen from four grades of flours with initial bromate levels of 15 or 30 p.p.m. ranged from 1 to 3.5 p.p.m. per hour. While these rates are considerably higher, especially for the nonfermenting doughs, than the rates of conversion of bromate to bromide shown in Table I, Cunningham and Anderson did point out that different flours would show different rates of bromate loss and that in fermenting doughs, mixing in air and punching and molding gave a lower rate of bromate loss as compared to dough mixed in nitrogen. With an initial bromate level of 15 p.p.m., the rates of loss of potassium bromate for fermenting doughs mixed in air and worked were reported (5) to be 0.95, 0.88, and 0.60 p.p.m. per hour, respectively, for patent, clear, and intermediate flours. These rates are in the same order of magnitude as the rate of 0.81 p.p.m. per hour for conversion of bromate to bromide in our fermenting dough with an initial potassium bomate level of 15 p.p.m. Such agreements appear to indicate that bromate loss as measured by Cunningham and Anderson in fermenting doughs may be largely due to conversion of bromate to bromide.

The present results also show that for the flour used in these experiments, the rates of conversion of bromate to bromide were decidedly greater in fermenting than in nonfermenting doughs. This is in agreement with the finding of Hlynka, Templin, and Anderson (10), who reported that yeast accelerated the decomposition of bromate in dough. It is possible that products of fermentation may be reducing certain amounts of bromate over and above the quantities reduced in nonfermenting doughs. Further experiments, using labeled bromate, are under way in an attempt to shed some light on this point. Preliminary tests with ordinary analytical techniques do appear to indicate that some bromate was lost when kept in an aqueous mixture containing yeast, sugar, salt, phosphate, and malt. When 20 mg. of potassium bromate were kept in 100 ml. of 3% yeast suspension for different lengths of time followed by determinations of bromate using an iodometric method (addition of excess potassium iodide and then titration of the iodine liberated with standardized sodium thiosulfate), there were no significantly large changes in the amount of iodine liberated over a period of 4 hours. On the other hand, when 20 mg. of potassium bromate were placed in 100 ml. of an aqueous mixture containing

yeast, sugar, salt, ammonium dihydrogen phosphate, and malt in concentrations as used in our baking formula, iodometric titrations showed an immediate decrease of iodine liberated, corresponding to about 3.5 mg. of potassium bromate. This was followed by a more gradual decrease in liberated iodine equivalent to a loss of 1.5 mg. of potassium bromate over a period of 4 hours. Perhaps the initial decrease was due to interaction of some of the liberated iodine with constituents of the fermenting mixture. The more gradual decline in liberated iodine over the 4-hour period, however, seemed likely to be the result of reduction of 1.5 mg. of potassium bromate by the fermenting mixture under the conditions of our test experiment.

The improver effect of the $Br^{82}$-labeled potassium bromate on loaf volumes is shown in Fig. 2. Total recoveries of the $Br^{82}$ activity and amounts of bromate converted to bromide in aqueous extracts of bread are given in Table II.

As compared to nonfermenting and fermenting doughs, total recoveries of $Br^{82}$ activity were lower in aqueous extracts of baked bread, with the amounts of unextractable $Br^{82}$ activity ranging up to 30%. Similar observations have been made in studies with baked bread containing $I^{131}$-labeled potassium iodate (4). The bread residues after extraction of iodate and iodide with ammoniacal methanol were reported to contain some 23 to 29% of the total $I^{131}$ activity.

For all levels of initial bromate studied, the amounts of bromate converted to bromide in the bread extracts were greater than the de-

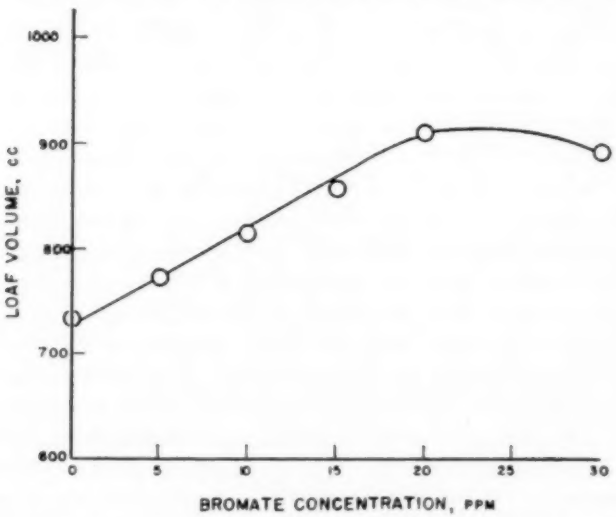


Fig. 2. Loaf volume response to increments of $Br^{82}$-labeled potassium bromate.

TABLE II

BROMINE-82 ACTIVITIES IN BAKED BREAD
(Expressed as percent of the bromate added initially)

| INITIAL BROMATE LEVEL | TOTAL RECOVERY IN AQUEOUS EXTRACT | BROMATE CONVERTED TO BROMIDE IN AQUEOUS EXTRACT |
|---|---|---|
| *ppm* | % | % |
| 5 | 86 | 56 |
| 10 | 92 | 69 |
| 15 | 79 | 53 |
| 20 | 85 | 65 |
| 30 | 70 | 46 |

grees of conversion in the fermenting doughs at the time when these doughs were ready for baking (after proofing), indicating, as one might expect, reduction of further quantities of bromate during baking. That fraction of the $Br^{82}$ activity in the bread extracts which was not bromide amounted to 20–30% of the initial bromate (figures in column 2 – figures in column 3 in Table II). This fraction was shown to contain some unchanged bromate, although it was reported in 1940 (14) that no bromate could be detected when the bread was taken from the oven. When all bromide ions have been removed as silver bromide from the bread extracts, addition of further quantities of silver ions gave precipitates of silver bromate which were radioactive. No direct comparisons between the activities of the silver bromate and our other experimental data were possible because of differences in self-absorption and sample geometry for samples prepared from solid silver bromate and samples prepared from drying aliquots of aqueous solutions. A rough estimation, however, did indicate that about one-half of the nonbromide fraction of the extracted $Br^{82}$ activity from bread could be accounted for as bromate. For example, for the bread baked with 20 p.p.m. labeled potassium bromate, solid samples of silver bromide and silver bromate, fractionally precipitated from the aqueous extract after addition of carriers, showed activities of 2960 and 346 counts per minute, respectively. If it were assumed that the sum of the silver bromide and silver bromate activities were equal to the total activity in the aqueous extract, i.e., 85% of the bromate initially added (Table II), the amount of unchanged bromate in the aqueous extract would be equal to $346/(346 + 2960) \times 85 = 9\%$.

Currently, it is generally accepted, from tests or titrations by ordinary chemical means, that there is no residual bromate in baked bread (9). The present findings are contrary to such a conclusion and appear to indicate that analytical tests by ordinary chemical means may not be

of sufficient sensitivity to detect unchanged bromate in the presence of other constituents extracted from baked bread. In this connection, it is of interest to note that Boyd and co-workers (2) have pointed out that the determination of bromate by micropotentiometric titration with 0.01*N* silver nitrate is believed to be reliable only down to a total bromine content of 20 p.p.m. Moreover, the work of Conn *et al.* (4) on the decomposition of labeled potassium iodate during the baking of bread has shown the presence of unchanged iodate in baked bread in amounts varying from 3.0 to 14.8% of the iodate initially added. Such evidence strongly supports the capability of the highly sensitive tracer technique in detecting unchanged bromate in the baked bread investigated in the present experiments.

In summary, the present results showed that for the flour used in these experiments, with initial potassium bromate levels of 5, 10, 15, 20, and 30 p.p.m., reduction of part of the bromate to bromide was observed in all cases for nonfermenting and fermenting doughs and for baked bread. The amount of bromate reduced to bromide was lowest in nonfermenting doughs and highest in the baked bread. For example, with an initial bromate concentration of 20 p.p.m., which gave the highest loaf volume for the baked bread, the amounts of bromate converted to bromide in 4 hours after mixing were about 1 and 4 p.p.m., respectively, for nonfermenting and fermenting doughs; while for the baked bread, the bromate reduced to bromide amounted to 13 p.p.m. While these observations do not constitute proof of any mechanism of improver action, the findings show unequivocally that some oxidation-reduction reactions have taken place and would, therefore, be consistent with mechanisms involving oxidation-reduction reactions.

It is known that, with added bromate, changes in physical properties occur in unleavened doughs (1, 6). That such changes also took place in our nonfermenting doughs was apparent from their handling properties. If the reduction of bromate to bromide was responsible for these changes in unleavened doughs, the results indicate that only a relatively small fraction of the total bromate (in the order of 1 p.p.m.) was involved. Taking into account the change in oxidation number of 6, the reduction of about 1 p.p.m. of potassium bromate to bromide would be equal to a reduction of the order of 0.04 equivalents per $10^6$ g. of flour. Recent determinations of sulfhydryl groups in wheat flour have given a value of about 0.2 equivalents of sulfhydryl groups per $10^6$ g. of flour for flours derived from the bread wheat varieties studied (11). If oxidation of sulfhydryl groups were involved in the improver action, reactions of 0.04 equivalents of sulfhydryl groups per $10^6$ g. of flour with bromate would amount to the oxidation of some

20% of the total sulfhydryl groups, which very probably would be sufficient to cause modifications in protein structures (13) or changes in the structures of a three-dimensional dough network (6).

**Acknowledgment**

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# THE VITAMIN CONTENT OF WHEAT, FLOUR, AND BREAD[1]

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## ABSTRACT

Evaluation of procedures for liberating vitamins from wheat and wheat products showed some to be inadequate. Hydrolysis conditions were worked out, where necessary, to give satisfactory values. The methods adopted were: 1) niacin, biotin, choline – autoclaving with 1*N* sulfuric acid for 1 hour at 20 lb. pressure; 2) inositol – autoclaving with 6*N* hydrochloric acid for 8 hours at 20 lb. pressure; 3) *p*-aminobenzoic acid – autoclaving with 1*N* sodium hydroxide for 15 minutes at 20 lb. pressure; 4) pantothenic acid – digesting with Mylase P in phosphate buffer; 5) folic acid – digesting with chicken pancreas in acetate buffer.

Four wheat blends, their flours, and breads prepared from the flours were assayed for nine vitamins. Levels of thiamine, riboflavin, niacin, pantothenic acid, and *p*-aminobenzoic acid were directly related to the protein level of the wheats. This relationship did not exist for biotin, choline, folic acid, and inositol. The spring wheat blends were higher in vitamins (and protein) than were winter wheats. Choline levels were least affected by milling and by the breadmaking procedure. Except for choline, vitamin levels on an equal moisture basis were higher in the breads than in their respective flours because of the contribution of other ingredients incorporated in the doughs. The increase was especially large for folic acid. Short-patent flour from a spring-wheat blend had vitamin levels comparable to those of long-patent flours from winter wheats.


Bailey (4) has reviewed the literature prior to 1943 on the vitamin content of wheat and flour. Hegsted, Trulson, and Stare (11) have summarized much of the more recent data. It is apparent that the vitamin content of wheat may vary considerably, depending upon the variety, climate, composition of the soil, harvesting, and handling procedures. Wheats commercially milled for bread flours are usually blends of several varieties from different areas.

This laboratory is involved in a study to determine the range of vitamin concentrations in commercial wheat blends, bread flours milled from these blends, and of the breads baked from the flours. This paper presents the methods selected for use in this study and the values obtained on four series of wheat blends, flours, and breads. The determination of vitamin $B_6$ was found to pose special problems and will be presented separately.

## Materials and Methods

*Sample Description.* The samples employed were those used by Hepburn *et al.* (12) in their studies of amino acids. Sample descriptions

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are repeated in Table I for convenience. The breads were prepared by the sponge-dough procedure using a formula representative of commercial practice. The following ingredients were used per kg. of flour: compressed yeast, 25 g.; yeast food, 5 g.; sodium chloride, 20 g.; sucrose, 60 g.; lard, 30 g.; calcium propionate, 1.1 g.; nonfat dry milk, 40 g.; and water, 680 g. An amount of a commercial enrichment wafer was added during the dough stage to meet the requirements of the Standards of Identity for enriched white bread. One wafer supplied 190 mg. thiamine hydrochloride, 70 mg. riboflavin, 1200 mg. niacin, and 900 mg. iron per 100 lb. of flour. One-pound loaves were baked at an oven temperature of 450°F. (232°C.) for 23 minutes.

TABLE I

SAMPLE DESCRIPTION SUPPLIED BY MILLS
14% Moisture basis

| Series | Wheat Class and Source | Test Weight | Flour Yield | Flour Patent |
|---|---|---|---|---|
| | | lb | % | % |
| 1 | Hard Red Spring — Montana, North Dakota, South Dakota, Minnesota | 57.8 | 71.8 | 98.5 |
| 2 | | 58.0 | 72.2 | 80.0 |
| 3 | Hard Red Winter — Kansas, Colorado | 61.5 | 73.7 | 95.0 |
| 4 | | 61.0 | 74.4 | 96.0 |

The breads were sliced and dried in air at 74° – 78°F. (23.3° – 25.6°C.). The wheats and breads were ground in a hammer mill to pass a screen with 0.024-in. openings. All samples were protected from light during handling and were stored at −20°F. (−28.9°C.) until needed.

*General Approach to Assays.* A search of the literature revealed that many different hydrolysis and assay procedures have been used for some of the vitamins. In many cases the adequacy of a method was not tested for each product analyzed. To investigate methodology, hydrolysates based upon some of the methods given in the literature were prepared of wheat, flour, and bread. All appropriate hydrolysates were assayed for each vitamin, whereby a broad range of hydrolysis conditions was screened. Many of the conditions were readily eliminated because of incomplete liberation of the vitamin, or because of excessive vitamin destruction. It was also determined whether a hydrolysate could be assayed for more than one vitamin. Where necessary, more intensive studies were made.

*Biotin. Lactobacillus arabinosus* 17–5 and the medium of Wright and Skeggs (23) were used. The assays were incubated at 30°C. as

recommended by Barton-Wright (6). Lower values resulted when wheat or flour was autoclaved at 20 lb. pressure for 30, 60, or 120 minutes with 6*N* sulfuric acid than when 0.5*N*, 1*N*, or 2*N* sulfuric acid was used. Biotin values for bread were unaffected by acid concentration within these limits. Hydrolysis with 50 ml. of 1*N* sulfuric acid per g. of sample for 1 hour at 20 lb. pressure with subsequent removal of sulfate as the barium salt proved satisfactory for all of these products. The same hydrolysates could be used for choline and microbiological niacin determination.

*Choline. Neurospora crassa* M34486 and the basal medium of Horowitz and Beadle (15) were used. Hydrolysates prepared by autoclaving samples with 1*N*, 2*N*, 3*N*, 4*N*, or 6*N* sulfuric acid for 1 hour at 20 lb. pressure gave similar choline values. The hydrolysis procedure selected was the same as for biotin. Since additions of DL-methionine to quadruple the methionine levels of the samples did not interfere with the assay, it was unnecessary to separate choline from methionine. This is consistent with the findings of Horowitz and Beadle (15) and of Hodson (14).

*Niacin. Lactobacillus arabinosus* 17–5 and the basal medium of Krehl, Strong, and Elvehjem (18) as modified by Barton-Wright (5) were used. The hydrolysates were prepared as described for biotin analysis.

*Pantothenic Acid. Lactobacillus arabinosus* 17–5 and the basal medium proposed by Hoag *et al.* (13) were used. Samples were digested with 10% by weight of Mylase P[2] in acetate buffer under toluene for 24 hours at 37°C. as suggested by Ives and Strong (16). This gave higher (10–20%) and more consistent results than did autoclaving with water according to the procedure of Strong *et al.* (21) or digesting with the pigeon liver-alkaline phosphatase extract of Neilands and Strong (20).

*Folic Acid. Streptococcus faecalis* R and the basal medium of Capps, Hobbs, and Fox (8), modified to contain citrate instead of acetate, were used. One-gram samples were digested under toluene with 20 mg. of dehydrated chicken pancreas[3] in 10 ml. of 0.2*M* phosphate buffer (pH 7.0) for 24 hours at 37°C. (2). It was found necessary to double the prescribed quantity of buffer because of the dry and starchy nature of the samples.

*Inositol.* The method of Beadle (7) using *Neurospora crassa* M37401 gave satisfactory and reproducible results. Use of *Saccharomyces carlsbergensis* and the basal medium of Atkin *et al.* (3) as modified by

[2] Wallerstein Laboratories, New York, N.Y.
[3] Difco Laboratories, Detroit, Michigan.

Johnson (17) did not prove to be a reliable method for these products. Ranges of inoculum strength of 0.005–0.150 mg. of wet yeast per ml. and incubation times of 12–30 hours were tested. The assays were incubated at 30°C. in 125-ml. Erlenmeyer flasks. Marked downward drift was encountered, which was not caused by the potassium chloride formed upon neutralization of the hydrolysates with potassium hydroxide. Experiments showed no adverse effects due to potassium chloride at final concentrations below 0.3*N*.

It is well known that prolonged hydrolysis with strong acid is necessary to free inositol from cereal products for subsequent assay. In this study samples were autoclaved with 10 ml. of 6*N* hydrochloric acid per g. for 8 hours at 20 lb. pressure. Comparable values were obtained on 12-hour hydrolysates.

*Para-Aminobenzoic Acid.* Except for the extraction procedure, the method of Agarwala and Peterson (1), using the assay organism *Neurospora crassa* M1633 of Tatum and Beadle (22), was employed. Agarwala and Peterson recommended that samples be prepared for assay by autoclaving with water. They showed that acid or alkaline hydrolysis of some yeasts and a liver extract gave higher values than did water extraction, but attributed the increase to the liberation of *p*-aminobenzoic acid from folic acid. Water extraction proved inadequate for liberating *p*-aminobenzoic acid from wheat, flour, and bread. Values obtained on hydrolysates prepared by several methods are given in Table II. Hydrolysis with 1*N* sodium hydroxide for 15 min-

TABLE II

PARA-AMINOBENZOIC ACID VALUES OBTAINED WITH VARIOUS HYDROLYSIS PROCEDURES

Dry-weight basis

| REAGENT[a] | NORMALITY | TIME | WHEAT | FLOUR | BREAD |
|---|---|---|---|---|---|
| | | *hours* | *γ/g* | *γ/g* | *γ/g* |
| Water | .. | 0.25 | 0.0 | 0.0 | 0.72 |
| Water | .. | 2.0 | 0.62 | 0.0 | 0.41 |
| Water | .. | 12.0 | 1.03 | 0.16 | 0.70 |
| Sodium hydroxide | 0.1 | 0.25 | 5.75 | 0.76 | 1.34 |
| Sodium hydroxide | 1.0 | 0.25 | 5.98 | 0.87 | 1.32 |
| Sodium hydroxide | 1.0 | 0.25 | 6.05[b] | 0.86[b] | 1.21[b] |
| Sodium hydroxide | 1.0 | 0.5 | 5.80 | 0.88 | 1.13 |
| Sodium hydroxide | 1.0 | 1.0 | 5.71 | 0.92 | 1.22 |
| Sodium hydroxide | 1.0 | 2.0 | 5.88 | 0.85 | 1.28 |
| Sodium hydroxide | 2.0 | 0.25 | 6.08 | 0.92 | 1.14 |
| Sodium hydroxide | 2.0 | 2.0 | 5.72 | 0.86 | 1.13 |
| Sulfuric acid | 0.055 | 4.0 | 0.0 | 0.0 | 0.0 |
| Sulfuric acid | 1.0 | 1.0 | 3.46 | 0.53 | 0.85 |
| Sulfuric acid | 1.0 | 4.0 | 0.0 | 0.0 | 0.0 |

[a] One-gram samples were autoclaved at 20 lb. pressure with 25 ml. of reagent.
[b] Before autoclaving, 0.5, 0.1, and 0.4 γ folic acid added per g. of wheat, flour, and bread, respectively.

TABLE III

VITAMIN AND PROTEIN CONTENT OF FOUR SERIES OF WHEAT, FLOUR, AND BREAD
Values expressed on dry-weight basis

| | Wheat Series | | | | Flour Series | | | | Bread Series | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Protein[a], % | 17.36 | 15.44 | 14.12 | 13.86 | 16.81 | 13.91 | 13.18 | 13.01 | 16.63 | 13.89 | 13.39 | 13.27 |
| | *mg/100 g* | | | | *mg/100 g* | | | | *mg/100 g* | | | |
| Thiamine[b] | 0.44 | 0.41 | 0.41 | 0.34 | 0.15 | 0.085 | 0.096 | 0.086 | 0.49 | 0.42 | 0.47 | 0.45 |
| Riboflavin | 0.20 | 0.16 | 0.15 | 0.14 | 0.044 | 0.034 | 0.033 | 0.028 | 0.29 | 0.29 | 0.28 | 0.28 |
| Niacin | 7.42 | 7.33 | 6.70 | 6.34 | 1.62 | 1.30 | 1.35 | 1.23 | 4.66 | 4.43 | 4.24 | 4.24 |
| Biotin | 0.018 | 0.019 | 0.014 | 0.014 | 0.0026 | 0.0020 | 0.0020 | 0.0018 | 0.0033 | 0.0028 | 0.0028 | 0.0028 |
| Choline[c] | 224.0 | 223.0 | 215.0 | 204.0 | 208.0 | 211.0 | 206.0 | 206.0 | 212.0 | 209.0 | 201.0 | 188.0 |
| Pantothenic acid[d] | 1.62 | 1.46 | 1.21 | 1.20 | 0.65 | 0.55 | 0.59 | 0.57 | 0.75 | 0.62 | 0.70 | 0.69 |
| Folic acid | 0.049 | 0.053 | 0.047 | 0.046 | 0.013 | 0.010 | 0.012 | 0.010 | 0.040 | 0.039 | 0.040 | 0.041 |
| Inositol | 371.0 | 383.0 | 372.0 | 352.0 | 63.0 | 41.0 | 44.0 | 41.0 | 69.0 | 46.0 | 50.0 | 46.0 |
| *p*-Aminobenzoic acid | 0.63 | 0.57 | 0.46 | 0.39 | 0.080 | 0.042 | 0.041 | 0.035 | 0.120 | 0.083 | 0.085 | 0.080 |

[a] Kjeldahl N × 5.7.
[b] As thiamine hydrochloride.
[c] As choline chloride.
[d] As calcium pantothenate.

TABLE IV

RELATIVE CHANGES IN VITAMIN LEVELS DURING THE CONVERSION OF WHEAT TO BREAD; PERCENT GAINED OR LOST ON THE DRY-WEIGHT BASIS

| | Milling Change[a] (Wheat to Flour) | | | | Baking Change (Flour to Bread) | | | | Net Change (Wheat to Bread) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Series 1 | Series 2 | Series 3 | Series 4 | Series 1 | Series 2 | Series 3 | Series 4 | Series 1 | Series 2 | Series 3 | Series 4 |
| Thiamine | −66.5 | −79.4 | −76.4 | −74.6 | +234 | +392 | +388 | +426 | +11.9 | + 1.40 | +15.3 | +33.3 |
| Riboflavin | −78.1 | −79.3 | −77.7 | −80.6 | +552 | +744 | +761 | +911 | +42.8 | +75.0 | +91.9 | +96.5 |
| Niacin | −78.2 | −82.3 | −79.8 | −80.6 | +188 | +241 | +214 | +245 | −37.2 | −39.6 | −36.7 | −33.1 |
| Biotin | −85.6 | −89.5 | −85.7 | −87.1 | + 26.7 | + 40.0 | + 40.0 | + 55.6 | −81.7 | −85.3 | −80.0 | −80.0 |
| Choline | − 7.1 | − 5.4 | − 4.2 | + 1.0 | + 1.9 | − 0.9 | − 2.4 | − 8.7 | − 5.4 | − 6.3 | − 6.5 | − 7.8 |
| Pantothenic acid | −59.9 | −62.5 | −50.9 | −52.8 | + 16.0 | + 14.0 | + 18.4 | + 22.6 | −53.4 | −57.2 | −41.9 | −42.2 |
| Folic acid | −74.5 | −81.1 | −74.5 | −78.3 | +208 | +290 | +233 | +310 | −18.4 | −26.4 | −14.9 | −10.9 |
| Inositol | −83.1 | −89.1 | −88.5 | −88.3 | + 10.7 | + 12.1 | + 15.3 | + 12.4 | −81.3 | −87.9 | −86.4 | −86.9 |
| *p*-Aminobenzoic acid | −87.4 | −92.7 | −91.0 | −91.1 | + 52.5 | + 97.6 | +107 | +128 | −80.7 | −85.5 | −81.4 | −79.7 |

[a] See Table I for flour yield and patent.

utes at 20 lb. pressure was selected for use in this study. The sodium chloride formed upon neutralization with hydrochloric acid did not interfere with the assay.

Folic acid, when added to samples before hydrolysis in amounts calculated to double the levels already present, did not increase *p*-aminobenzoic acid values (Table II). These amounts of folic acid, when subjected to the same hydrolysis and assay conditions in the absence of sample, produced no measurable response for *p*-aminobenzoic acid.

*Thiamine and Riboflavin.* These vitamins were determined only by chemical methods (2).

## Results and Discussion

Results of the determination of vitamins in the four series of wheats, flours, and breads are given in Table III.

In general, the vitamin content of the wheat blends increased with increasing amounts of protein. This was more marked for thiamine, riboflavin, niacin, pantothenic acid, and *p*-aminobenzoic acid than it was for biotin, choline, folic acid, and inositol. Despite the lower protein level of wheat 2, its content of biotin, folic acid, and inositol was similar to that of wheat 1. Spring wheat blends 1 and 2 were higher in all vitamins and in protein than were winter wheat blends 3 and 4, except for a small statistically insignificant reversal for inositol in wheats 1 and 3. Many of these differences were small.

Relative changes in vitamin content on milling and baking are given in Table IV. The losses of choline during milling of wheat to flour averaged about 4%. These results are not in agreement with Engel (9), who found that flour contained 50% less choline than wheat. Glick (10) reported choline losses of 20–30% during milling of wheat to flour. These workers extracted the samples with methanol, hydrolyzed the extract by aqueous barium hydroxide, and determined choline as the reineckate.

Milling losses, including bleaching, were high for all other vitamins. These losses ranged from 51% for pantothenic acid to 93% for *p*-aminobenzoic acid. Short-patent flour 2, milled from spring wheat blend 2, had vitamin levels comparable to long-patent flours 3 and 4, milled from the lower-protein winter wheat blends 3 and 4. The vitamin levels in the flours and breads appeared to be less related to protein content than the wheat blends were.

The baking change given in Table IV includes the effects of ingredient addition, fermentation, and baking. The increases in thiamine, riboflavin, and niacin levels were the result of enrichment supplementation. Small reductions in choline occurred in breads 2, 3, and

4, but these were not statistically significant. All other vitamins showed increases which ranged from 1.9% for choline in series 1 to 310% for folic acid in series 4.

Net changes from wheat to bread, expressed on the dry-weight basis, ranged from a gain of 96.5% in riboflavin for series 4 to a decrease of 87.9% in inositol in series 2. Largest decreases (approximately 80–88%) occurred in biotin, inositol, and *p*-aminobenzoic acid. Pantothenic acid levels were lowered about 50%. Decreases were much less for folic acid (10.9–26.4%) and were lowest for choline (5.4–7.8%).

*Statistical Treatment of Sample Results.* Data obtained by microbiological assays were subjected to analysis of variance between series. The mean-square values are given in Table V. The chemical determinations for thiamine and riboflavin were not performed in replicate because the literature is replete with analytical values for these vitamins in wheat and wheat products. They therefore could not be analyzed statistically.

TABLE V

ANALYSIS OF VARIANCE BETWEEN SAMPLE SERIES[a]

(Mean squares)

| | Replicate Assays | Between Wheats | Between Flours | Between Breads |
|---|---|---|---|---|
| Niacin | 3 | 0.811** | 0.090** | 0.119** |
| Biotin | 2 | 0.137** | 0.244** | 0.095* |
| Choline | 3 | 0.020* | 0.004 | 0.039* |
| Pantothenic acid | 3 | 0.129* | 0.006* | 0.849** |
| Inositol | 2 | 0.032 | 0.022** | 0.024** |
| *p*-Aminobenzoic acid | 2 | 2.39 ** | 7.96 ** | 0.082** |

[a] No significant differences were found in case of folic acid. The variance was 0.00028, 0.00005, and 0.00021 for wheats, flours, and breads, respectively. Two replicate assays were performed.

For the different products within each series the differences for most vitamins are so great that statistical analysis is not necessary. For example, the biotin content of the four wheats averages 0.016 mg. per 100 g.; that of the flours averages 0.0021 mg. per 100 g.; and that of the breads averages 0.0029 mg. per 100 g. Doubtful differences were tested for significance with the following results: 1) choline in wheat versus flour, 1% level; 2) choline in flour versus bread, no significant difference at the 5% level; 3) choline in wheat versus bread, 5% level; 4) folic acid in wheat versus bread, 1% level; 5) inositol in flour versus bread, 1% level.

It is difficult to assay the significance of the changes in levels of some of these nutrients due to processing. In case of thiamine, riboflavin, and niacin, sufficient knowledge concerning requirements is available to permit the Food and Nutrition Board of the National

Research Council (19) to publish recommended allowances for these nutrients. Distribution of these three vitamins in our food supply has been carefully studied and suboptimal intakes in segments of the population have been determined. The loss of these nutrients due to milling has been minimized by the flour and bread enrichment program. Recommended allowances have not been established for the other vitamins reported in this paper, nor is there any evidence of dietary insufficiencies that might be attributed to processing losses of foods made from wheat. More knowledge of human requirements and dietary sources is needed before many of these data can be evaluated in terms of human nutrition.

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# CAROTENOID CONTENT OF THE GRAIN FROM YELLOW ENDOSPERM-TYPE SORGHUMS[1]

C. W. BLESSIN, C. H. VANETTEN, AND RICHARD WIEBE

## ABSTRACT

Methods for estimating total carotenes and total xanthophylls and for isolating and identifying the major carotenoids were adapted for use on grain sorghum. The grain of common varieties of sorghum contained about 1.5 p.p.m. of total carotenoids. Crosses obtained in preliminary plant breeding of common varieties with "African yellow endosperm" varieties contained carotenoids as high as 8 to 9 p.p.m. Carotenoid content was higher when the seeds were protected from the weather after pollination. Major carotenoids present were identified as lutein, zeaxanthin, and beta-carotene. Other carotenoids present in small amounts in both feed sorghum and yellow corn, which were used for comparison purposes, were described but not positively identified. Carotenoids found in yellow corn but not detected in the grain sorghum were cryptoxanthin, hydroxy-alpha-carotene, and alpha-carotene.

Yellow fat-soluble pigments are important in feed grains, not only because of their vitamin A content but also because of the yellow color they impart to the skin and eggs of poultry and to fat of beef cattle. Preliminary work by the plant breeder has produced crosses of domestic feed sorghums and African yellow endosperm varieties which are higher in total carotenoids than common domestic varieties of the grain. To obtain information on the improvement made by plant

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breeders, present analytical methods were adapted to determine accurately the carotenoid content of grain sorghum. As far as is known, this information is the first to be published on the carotenoid content of grain sorghums.

## Analytical Procedure

*Materials.* Samples of crosses of African yellow endosperm with domestic varieties of feed sorghum were obtained from the Nebraska Agricultural Experiment Station at Lincoln. These crosses were grown during the 1956 and 1957 crop years. To study the effect of weathering, heads of alternate plants of some of the crosses were covered with paper bags immediately after pollination. Other grains examined were commercial grades of yellow milo, white Kafir, and yellow corn (Holmes 39, 1956 crop). All samples were ground to pass a 60-mesh screen (0.0098-in. opening).

*Quantitative Estimation of Total Carotenes and Total Xanthophylls.* Cooley and Koehn's (3) method of extraction was adapted to remove carotenoids from grain sorghum. Twenty or forty grams of sample, depending on the amount of carotenoids expected to be present, were used in each determination. After removal of the mixed solvents used in the extraction, the carotenoids were dissolved in a hexane-acetone solution and separated into carotene and xanthophyll fractions by selective elution from a magnesium oxide-celite column similar to the procedure described by Bickoff *et al.* (2) for the analysis of carotenoids in alfalfa meal.

The eluates were made up to a convenient volume and the carotenoids measured in a Beckman Model B spectrophotometer.[2] Carotenes were measured at 451 m$\mu$ and xanthophylls at 445 m$\mu$. Absorptivities of 251 and 231 were used for the carotene and xanthophyll fractions, respectively.

*Separation and Examination of the Carotenoids.* In addition, the carotenoids were separated by adsorption chromatography and then characterized by spectrophotometry.

Whole grain was extracted with the Soxhlet extractor for 30 minutes with absolute ethanol prior to being ground in order to remove the wax coating of the pericarp. Although some yellow pigmentation was removed by the extraction, absorption data revealed no carotenoid characteristics. An 80-g. sample of the ground dewaxed grain was extracted with methanol. Twenty-gram portions were put in each of four 250-ml. centrifuge bottles and 100 ml. of absolute methanol added.

[2] Mention of trade products or firm names does not imply that they are endorsed or recommended by the U. S. Department of Agriculture.

The air space in each bottle was flushed with nitrogen; the bottles were put on a shaker, covered with a black cloth, and shaken for 15 minutes. After shaking, the bottles were centrifuged to obtain a clear supernatant liquid. After removal of the supernatant, the extraction procedure was repeated until no color was visible in the methanol extract. The combined methanol extracts were reduced to about 125 ml. on a rotary evaporator.

About 125 ml. of absolute ethanol were added and the sample saponified by Goodwin's procedure (6). After the ether extract from this procedure was dried with anhydrous sodium sulfate, the ether was removed with the rotary evaporator at 30° to 40°C. The residue was taken up in hexane-diethyl ether (95:5). This mixture was more effective than 100% hexane in dissolving the residue, which probably contained nonsaponifiable material. The carotenoids contained in the extract were then separated by adsorption chromatography. Procedures developed by others (1,6) were used on yellow endosperm crosses and a sample of yellow corn for comparison purposes.

Early work on extracts that were not saponified gave a greater percentage of carotenoids in the lower chromatographic bands. These bands were not as well defined. This fact suggested to the authors that esters of the hydroxy carotenoids (4) may be present in the native material which would adsorb further down the column. For this reason, all samples examined chromatographically were saponified.

## Results and Discussion

*Quantitative Estimation.* A 2-hour extraction gave maximum carotene and xanthophyll values for the grain sorghum. Four- and 7-hour extractions gave progressively less carotenes and xanthophylls up to a total loss of 6% in the 7-hour extraction. This loss in carotenoids was attributed to their destruction or to *cis*-isomer formation (9) in the hot solvent mixture. Recovery of total carotenoids from known solutions of carotenes and xanthophylls carried through the chromatographic separation on nine determinations averaged 94.4% with a standard deviation of 2.1. No loss of carotenoids was obtained during the removal of the mixed solvent with the rotary evaporator and their re-solution in hexane and acetone. Those water-soluble pigments, extracted with the mixed solvent and redissolved in hexane-acetone, were retained at the top of the magnesium oxide-celite column as a gray band.

Analytical values obtained on several commercial samples of grain sorghum and of randomly selected crosses with the yellow endosperm variety are shown in Table I. Analytical results show that crossing

TABLE I

CAROTENE AND XANTHOPHYLL CONTENT OF VARIOUS SORGHUM GRAINS

| VARIETY | CAROTENES | XANTHOPHYLLS |
|---|---|---|
| | *ppm* | *ppm* |
| Yellow milo No. 1 grade | 0.3 | 1.3 |
| Yellow milo No. 1, loose glumes removed | 0.2 | 0.9 |
| Yellow milo No. 3 | 0.2 | 1.1 |
| White Kafir No. 1 | 0.2 | 1.1 |
| Yellow endosperm cross, sample 1 | 0.5 | 4.2 |
| Yellow endosperm cross, sample 2 | 0.3 | 2.5 |
| Yellow endosperm cross, sample 3 | 1.1 | 4.5 |

with the yellow endosperm variety increases the carotenoid content markedly. The carotenoid nature of the small amount of color obtained from the commercial varieties was confirmed by their typical carotene and xanthophyll absorption curves. Determination of carotenoid content from bagged and open samples showed clearly that it increased when the seed head was protected from weathering, as shown in Table II.

TABLE II

CAROTENOID CONTENT OF YELLOW ENDOSPERM CROSSES EXPOSED AND PROTECTED FROM WEATHERING

| NUMBER | CAROTENES[a] | | XANTHOPHYLLS[a] | |
|---|---|---|---|---|
| | Bagged | Open | Bagged | Open |
| | *ppm* | *ppm* | *ppm* | *ppm* |
| 1956 Crop — 1 | 0.9 | 0.4 | 5.3 | 3.3 |
| 2 | 1.2 (1.0) | 0.3 (0.5) | 6.7 (6.2) | 2.8 (2.7) |
| 3 | 1.4 (1.2) | 0.5 (0.4) | 7.0 (6.8) | 3.5 (3.4) |
| 4 | 0.7 | 0.6 | 4.8 | 4.1 |
| 1957 Crop — 1 | 1.4 | 1.1 | 7.6 | 4.9 |
| 2 | 1.5 | 0.8 | 8.3 | 4.6 |
| 3 | 1.0 | 0.5 | 5.8 | 3.7 |

[a] Figures in parenthesis in these columns are the values obtained for total carotenes and total xanthophylls by adding the carotenoid content of each band in the separation procedure. Band II and the beta-carotene band (Fig. 1) were considered as carotenes and the remaining bands as xanthophylls.

Table II also shows good agreement between the values obtained by the quantitative estimation method and those obtained by the chromatographic separation method. Complete removal of the carotenoids from the grain by both methods was assumed because no increase in carotenoid content was obtained by further extraction with fresh solvent.

*Identity of Carotenoids.* Figure 1 shows the major bands separated on the magnesium oxide-celite column from yellow endosperm grain sorghum crosses and those separated from yellow corn. The yellow corn contained alpha-carotene, hydroxy-alpha-carotene, and cryptoxan-

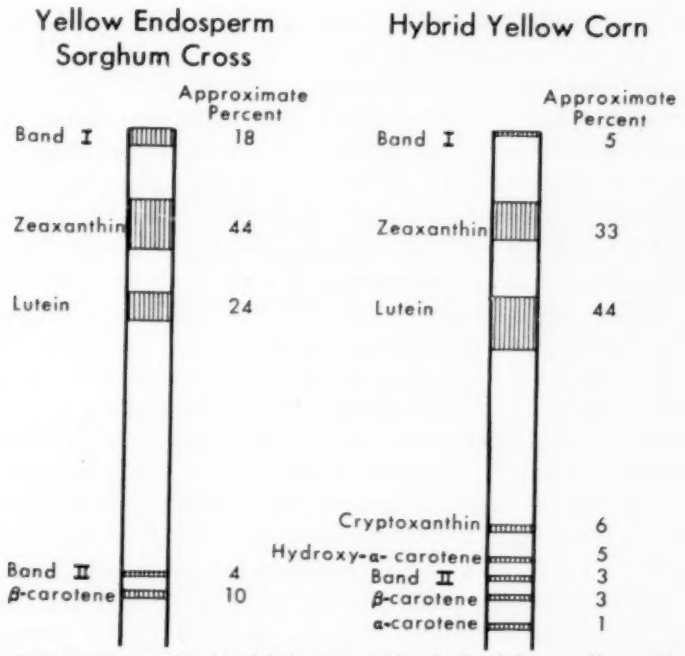


Fig. 1. Chromatographic bands of carotenoids obtained from yellow endosperm sorghum crosses and yellow corn developed on magnesium oxide-celite columns.

thin which were not found in the yellow endosperm crosses. Bands I and II appeared to be the same in both materials. Approximate percentages of the carotenoids present in each band were about the same from four different yellow endosperm crosses, regardless of whether they were protected from the weather or whether they were low or high in total carotenoids. Total carotenoid content of the yellow corn sample examined was 22 p.p.m. as compared with the values obtained for various yellow endosperm crosses given in Table II.

Identification of known carotenoids was established by the relative position of the bands on the adsorption column, their absorption spectra, and behavior on rechromatography. The bands corresponding to zeaxanthin and lutein were further identified by their rechromatography with known lutein and zeaxanthin. The cryptoxanthin and hydroxy-alpha-carotene from the yellow corn were tentatively identified, by comparison of their position on the chromatographic column and their absorption curves, with values reported in the literature (6).

Rechromatography of zeaxanthin on calcium hydroxide or sucrose and lutein on sucrose gave from two to four bands which behaved as stereoisomers. The absorption curve in the near ultraviolet (330 $m\mu$)

showed a typical *cis* peak in the case of those bands showing a minor shift of the main peaks to the lower wave lengths. These changes in the absorption curve agree with those reported by Zechmeister (10) for the different *cis* isomers of lutein and zeaxanthin. The *cis* isomers had a greater affinity for the chromatographic column. Rechromatography of any single band a second time on the same kind of column with the same elutriant again gave two to four bands of the isomers.

Rechromatography of beta-carotene gave one band with an absorption curve typical of the all-*trans* form, except in the case of the weathered yellow endosperm samples in which a second band comprising 20 to 30% of the optical density of the original band preceded beta-carotene. Its absorption curve gave a single broad peak at 445 $m\mu$ which did not resemble the absorption curve of alpha-carotene.

Bands I and II, which were not clearly identified with well-known carotenoids, were found in both yellow endosperm crosses and yellow corn. Some of the pigments in these bands may be partially oxidized carotenes or xanthophylls formed during processing or extraction.

Band II was separated into two bands by rechromatography on a long calcium hydroxide-celite column. These two bands have the same absorption curves and show the same chromatographic behavior as zeta-carotene (upper band) and pigment X (lower band), described by Goodwin and Osman (6,7). Such experimental evidence supports the statement of Goodwin (6) that K-carotene isolated from corn by Fraps and Kemmerer (5) is a mixture of zeta-carotene and pigment X.

Rechromatography of band I on a sucrose-celite column gave two bands. Further chromatography of the upper band gave several bands with similar absorption data. These carotenoids appeared to be isomers of a hydroxy derivative of alpha-carotene-5,6-epoxide. The absorption data, position on the column, and solubility properties agreed with trollixanthin, a derivative of lutein-5,6-epoxide (6, 8).

Further chromatography of the lower band from band I gave several bands with similar absorption data. The formation of *cis* isomers was indicated by a slight shift to the lower wave length of the maximum associated with the development of a *cis* peak at 330 $m\mu$. The absorption data of these isomers agreed with the data obtained on pigment X from band II.

Introduction of hydroxyl groups into the carotenoids has little effect on the wave-length position of the maxima, but the chromatographic adsorptive power is increased (compare beta-carotene and zeaxanthin). On the basis of this generalization, these isomers appeared to be a hydroxy derivative of pigment X. This relationship could also be

explained by pigment X being an ester, not room temperature saponified, of the above isomers (4).

The carotenoids in band I have not been previously reported, to our knowledge, as constituents of yellow corn.

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# THE EFFECT OF SUCROSE ON GLUTEN DEVELOPMENT AND THE SOLUBILITY OF THE PROTEINS OF A SOFT WHEAT FLOUR[1]

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## ABSTRACT

Additions of 15 and 30% sucrose (flour basis) to soft wheat flour increased dough development time in the mixograph. Sucrose levels of 60 and 120% prevented dough formation; this was attributed to the competition between sucrose and the gluten proteins for water.

Recovery of flour fractions by acetic acid extraction was not affected by the four concentrations of sucrose. Recovery of gluten protein diminished and soluble protein increased when sugar was present in the flour batter.

The percentage of soluble protein extractable from gluten dispersions in water and sucrose solutions decreased with an increase in the protein concentration in the medium and increased with increases in sucrose concentration. Peptization was greater at 25°C. than at 100°C.


Soft wheat flour is generally used to reduce the effect of gluten in chemically leavened batters and doughs. Sugar is frequently an ingredient of these products and is added for its tenderizing effect as well as for sweetness. Several mechanisms have been postulated to explain the observed tenderizing effect of sugars on flour products, but little experimental evidence has been published to support them.

One hypothesis often presented is that sugar tenderizes baked products because it peptizes the wheat protein. This probably stems from the work of Jago and Jago (4) in 1911, in which they found that the amount of gluten washed from a dough containing 20% sugar was less than that from a comparable dough prepared without sugar. They also reported that more gluten was dissolved by a 70% alcohol-sugar solution (containing 100 parts 70% alcohol and 20 parts sugar) than by 70% alcohol alone.

Pike and Johnson (5) attributed the tenderizing action of sugar in cakes to a weakening of the structure of the egg proteins and the starch gel. Hester, Briant, and Personius (3) found that, although sucrose reduced the hydration capacity of starch and weakened starch gels, it tended to increase the rigidity of flour gels, particularly those made from hard wheat flour.

There is need for basic information concerning the separate frac-

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tions of soft wheat flour and their function in determining the characteristics and quality of flour-sugar mixtures. The work described herein concerns the effect of sucrose on the gluten and soluble proteins of soft wheat flour.

## Materials and Methods

The flour used in this investigation was a straight-grade, unbleached sample milled from mixed strains of soft wheat grown in Pennsylvania and the surrounding area. The protein content of the flour was 8.1% (14% moisture basis).

*Mixing Curves.* Mixogram curves were made on a National Recording Micro-Mixer, using 35 g. of flour (14% moisture basis). Sucrose was added at levels of 0, 15, 30, 60, and 120%, and water was maintained at 55%, flour basis. The amount of flour was reduced to 30 g. for the 60% sugar doughs and to 25 g. for the 120% sugar doughs to adjust for the increased weight of the doughs containing the higher quantities of sugar. The mixograph was run for 10 minutes or more at a spring tension setting of 7.

*Flour Fractionation and Extraction of Protein.* The method adopted for fractionation was similar to that used by Sollars (6). It is essentially a water extraction of the flour prior to extraction with acetic acid. The water-solubles were extracted from 100 g. of flour combined with 0, 15, 30, 60, and 120 g. sucrose by stirring with 700 ml. of distilled water in a Hobart mixer for 1 hour, centrifuging, and decanting. The residual dough was dispersed in 700 ml. of 0.03*N* acetic acid for 5 minutes with a Waring Blendor, centrifuged, and decanted. The tailings and starch fractions were washed with distilled water and separated mechanically following centrifugation. The protein was precipitated from the acid dispersion by titrating with 5*N* sodium hydroxide to pH 5.8 while stirring continuously with a magnetic stirrer. The solution was centrifuged to recover the precipitated protein. The gluten and tailings were lyophilized and the starch air-dried. Only those water-solubles containing no sucrose were lyophilized.

Five 100-g. lots of flour containing each sucrose concentration were fractionated. The dried fractions from each of the five lots were ground to pass a 40-mesh sieve, combined, and treated as one sample. Total solids and nitrogen (Kjeldahl) of the dried fractions were determined by conventional methods. The nitrogen content of the water-solubles was determined for 100-g. aliquots concentrated under vacuum at 30°C. Recovery of the fractions and of the protein ($N \times 5.7$) was calculated.

Any gross alterations in the behavior of the flour components which may have been affected by sucrose and acetic acid fractionation were

observed by amylograms of the reconstituted gluten, starch, and tailings fractions of the flour. The flours were reconstituted using the fractions in the same ratio in which they were recovered.

Soluble protein was extracted from crude gluten obtained from the flour by the usual hand-kneading technique, lyophilized, and ground. The protein content of the crude gluten was 66.45%. The concentration of the dry gluten was adjusted so that the dispersion would contain 5, 2, or 1 g. of protein per 100 ml. of liquid used. The gluten was dispersed in 250 ml. of distilled water, 0.1*M*, 0.25*M*, or 0.5*M* sucrose solutions for 4 minutes in a Waring Blendor. The dispersion was transferred to a flask and, in one case, heated in a boiling-water bath; and in another, allowed to stand at room temperature, about 25°C. The heated dispersions were stirred mechanically for 1 hour, cooled to room temperature, swirled at half-hour intervals for 2 hours, and refrigerated (5°C.) for 24 hours. The dispersions treated at room temperature were swirled at half-hour intervals for 3 hours and refrigerated for 24 hours.

Each gluten dispersion was then centrifuged and filtered through Whatman No. 42 filter paper. The volume of the filtrate was measured and two 25-ml. aliquots were taken for nitrogen determinations. The soluble protein was calculated as the percentage of the total gluten protein.

## Results

*Mixing Characteristics and Gluten Development As Affected by Sucrose.* Curve A of Fig. 1 is the control curve for the soft wheat flour. Curves B, C, and D show the effects of the addition of 15, 30, and 60% sugar on the mixing characteristics of the flour. Curves for the 120% sugar doughs were identical with those for the 60% sugar doughs and are not shown. The most distinctive difference shown by the mixograms was the dough development time. Each increment of sucrose increased the mixing time. The development time for the control dough was 2.5 minutes. Upon the addition of 15 and 30% sucrose the dough development time increased to 6 and 12.5 minutes respectively. At the two highest sugar concentrations there was no development of a dough evident, even with 26 minutes of mixing.

The amplitude of the mixing curve of the control dough was greater and remained greater for a longer time than for any of the doughs containing sugar.

To investigate the effect of sucrose on the gluten of the flour as a causative factor in the increased dough development time and decreased amplitude in the mixing curves, gluten was prepared from 30

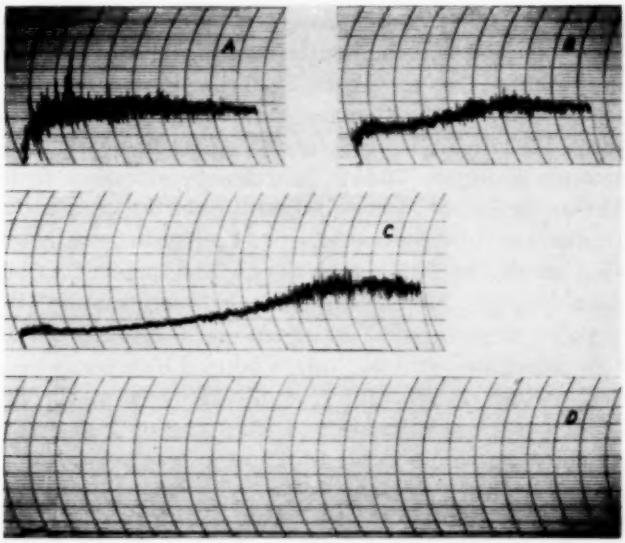


Fig. 1. Effect of sucrose concentration on mixogram patterns of soft wheat flour. A, Control, no sucrose; B, 15% sugar dough; C, 30% sugar dough; D, 60% sugar dough.

and 60% sugar doughs by four methods, described in Table I. Dried gluten prepared from the control doughs by the conventional method (1) averaged 2.29 g. from 25 g. of flour.

The presence of sugar in the dough at the time gluten development would normally occur, during kneading of hydrated gluten, resulted in a diminution in dried gluten obtained; 2.07 g. from the 30% sugar dough and 0.91 g. from the 60% sugar dough. When gluten was developed prior to the addition of sugar, however, the amount of dried

TABLE I

EFFECT OF METHOD OF PREPARATION ON THE YIELD OF GLUTEN FROM SUGAR DOUGHS[a]

| METHOD OF GLUTEN PREPARATION | SUCROSE CONCENTRATION[b] | | |
|---|---|---|---|
| | 0 | 30% | 60% |
| | g | g | g |
| A. Standard[c] | 2.3 | | |
| B. Sucrose added to dry flour; standard | | 2.1 | 0.9 |
| C. Kneaded flour-water dough 5 minutes before working in sucrose; standard | | 2.3 | 2.2 |
| D. Sucrose added to dry flour; kneaded dough 5 minutes; washed and centrifuged twice; then standard | | 2.3 | 1.6 |
| E. Same as D except kneaded additional 5 minutes following centrifugation | | | 2.2 |

[a] Mean of three samples from 25 g. flour (14% m.b.) dried at 100°C. 48 hrs.
[b] Flour basis.
[c] *Cereal Laboratory Methods* (1).

gluten recovered was approximately the same as that from the control dough. This suggested that the interference of sugar in gluten development was primarily due to a competitive action of both sugar and gluten for the water. To further test this hypothesis, doughs to which sucrose was added initially were kneaded, washed, and centrifuged twice to remove the sugar. The residual doughs were then hydrated for 1 hour. The average yield of dry gluten from the 30% sugar dough was 2.28 g., approximately the same as from the control dough. The low yield, 1.56 g., from the 60% sugar dough was believed to be due to limited development of hydrated gluten by manipulation. When the residual doughs were kneaded an additional 5 minutes following removal of the sugar, the yield was increased to 2.16 g.

Effect of sucrose concentration on the recovery of the flour fractions and the total protein by acetic acid extraction is shown in Table II. Acetic acid extraction was used because it was not possible to pre-

TABLE II

EFFECT OF SUCROSE ON THE YIELD AND PROTEIN CONTENT OF SOFT WHEAT FLOUR FRACTIONS EXRACTED BY WATER AND DILUTE ACETIC ACID

| Sucrose as Percent of Flour | Fraction | Fraction[a] Recovery | Protein (N × 5.7) (Dry Basis) | Distribution of Total Flour Protein Recovered[b] |
|---|---|---|---|---|
| | | % | % | % |
| 0 | Solubles | 5.3 | 0.2[d] | 15.5 |
| | Gluten | 7.0 | 81.3 | 59.9 |
| | Tailings | 24.2 | 2.1 | 5.3 |
| | Starch | 56.6 | 0.3 | 1.8 |
| | Total | 93.1 | | 82.5 |
| 15 | Solubles | [c] | 0.2[d] | 15.9 |
| | Gluten | 6.8 | 80.3 | 57.6 |
| | Tailings | 25.6 | 2.0 | 5.3 |
| | Starch | 56.8 | 0.3 | 1.6 |
| | Total | | | 80.4 |
| 30 | Soluble | [c] | 0.2[d] | 16.3 |
| | Gluten | 6.8 | 80.7 | 57.7 |
| | Tailings | 24.3 | 2.2 | 5.6 |
| | Starch | 58.7 | 0.3 | 1.6 |
| | Total | | | 81.2 |
| 60 | Soluble | [c] | 0.2[d] | 20.1 |
| | Gluten | 6.9 | 79.1 | 57.7 |
| | Tailings | 24.8 | 1.9 | 5.0 |
| | Starch | 58.0 | 0.3 | 1.7 |
| | Total | | | 84.5 |
| 120 | Solubles | [c] | 0.2[d] | 18.8 |
| | Gluten | 6.9 | 78.1 | 57.1 |
| | Tailings | 25.5 | 2.3 | 6.2 |
| | Starch | 57.6 | 0.3 | 1.7 |
| | Total | | | 83.8 |

[a] Percent of total flour solids, dry basis. Mean of five lots.
[b] Based on 9.45 g. total flour protein, dry basis.
[c] Total solids not determined.
[d] In 100-g. aliquot of water-solubles.

vent disintegration of sugar doughs by the usual kneading technique. Recovery of the solubles from the sugar batters was not determined, because of difficulties encountered in drying and because of the presence of sucrose in the flour solubles.

The quantity of gluten recovered from the flour (dry basis) was essentially the same, irrespective of the sucrose concentration. The amount ranged from 6.76% gluten recovery in the 30% sugar batter to 6.96% in the control batter. The 120% sugar batters yielded approximately the same amount of gluten as the control batters, 6.91%. The quality of gluten appeared the same irrespective of the sucrose concentration. In every instance the gluten was a cohesive and elastic mass.

While the starch and tailings were of lesser concern in this investigation, the yields are reported to calculate total recovery. The yield of these fractions was essentially unaffected by the presence of sucrose in any of the concentrations used.

The water-solubles from the control dough were lyophilized and subjected to a moisture determination; the total soluble solids recovered were 5.34% of the flour. The total yield of the fractions from the control batter was 93.07%. Part of the loss was mechanical and could not be avoided. Part may have been due to incomplete precipitation of the gluten proteins from the acetic acid dispersion.

The protein content of each of the fractions of the flour did not appear to be significantly affected by the addition of sucrose to the batters. The protein content of the gluten from 60 and 120% sugar batters was somewhat lower and the protein of the solubles was slightly higher than that of the same fractions from batters with 0, 15, and 30% sugar.

Only about 80 to 85% of the total flour protein was recovered. Of the amounts recovered under the same conditions, more gluten protein was obtained from the control batter than from any of the sugar batters. The concentration of sucrose used appeared to have little or no effect on the percentage of the gluten protein recovered.

The soluble protein recovered was least in the control batter, increased by less than 1% in the 15 and 30% sugar batters, and increased by 4.56 and 3.26% in the 60 and 120% sugar batters.

Amylograms of a 12% suspension of the unfractionated flour and flours reconstituted from the gluten, starch, and tailings fractions obtained from 0, 15, 30, 60, and 120% sucrose batters were essentially the same, as seen in Table III. The maximum and terminal paste viscosities of the reconstituted flours were slightly higher than that of the original flour. This is attributed to the absence of the water-solubles, which have been shown to depress the viscosity of heated flour suspensions (2).

TABLE III

AMYLOGRAPH CURVE CHARACTERISTICS OF SOFT WHEAT FLOUR AND RECONSTITUTED FLOUR FRACTIONS OBTAINED FROM SUGAR-FLOUR BATTERS BY ACETIC ACID EXTRACTION

| SUCROSE CONTENT OF BATTER BEFORE FRACTIONATION [a] | TEMPERATURE OF TRANSITION | MAXIMUM VISCOSITY | PASTE TEMPERATURE AT MAXIMUM VISCOSITY | VISCOSITY AFTER 5 MINUTES AT 93°C. |
|---|---|---|---|---|
| | °C | B.U. | °C | B.U. |
| Original flour [b] | 59.5 | 485 | 91.0 | 440 |
| Reconstituted flours [c] | | | | |
| 0 | 60.2 | 540 | 90.0 | 490 |
| 15 | 59.5 | 540 | 90.5 | 490 |
| 30 | 61.0 | 535 | 90.0 | 490 |
| 60 | 61.0 | 555 | 90.0 | 505 |
| 120 | 59.5 | 535 | 90.5 | 490 |

[a] Percent based on flour weight.
[b] 60 g. Flour (14% m.b.) and 440 g. water.
[c] Weight of starch, tailings, and gluten based on proportions obtained from the flour, 14% m.b.

These data indicate that the presence of sugar in a flour batter and its subsequent removal and the use of acetic acid in fractionation has no adverse effect on the gelatinization characteristics of the flour components.

*Soluble Protein and Gluten Dispersions as Affected by Sucrose.* The sucrose concentration, the protein concentration, and the temperature of the dispersion each affected the solubility of the gluten protein, as seen in Table IV. The differences among each of the three treatments was significant at the 1% level of probability as determined by analysis of variance.

Within any given protein level, 5, 2, or 1 g. per 100 ml., and at both 25° and 100°C., a gradual and increasing amount of protein was pep-

TABLE IV

PERCENT OF SOLUBLE PROTEIN EXTRACTED FROM GLUTEN PROTEIN BY WATER AND BY SUCROSE SOLUTIONS AT 25°C. AND 100°C.[a]

| PROTEIN CONCENTRATION [b] | SUCROSE CONCENTRATION | | | |
|---|---|---|---|---|
| | 0 | 0.10M | 0.25M | 0.50M |
| g/100 ml | | | | |
| | Extraction at 25°C. | | | |
| 5 | 7.2 | 8.3 | 9.3 | 11.5 |
| 2 | 15.8 | 17.3 | 18.3 | 21.9 |
| 1 | 19.9 | 21.8 | 24.7 | 26.8 |
| | Extraction at 100°C. | | | |
| 5 | 4.9 | 5.8 | 7.4 | 9.4 |
| 2 | 10.8 | 12.8 | 14.7 | 19.0 |
| 1 | 16.6 | 17.2 | 21.8 | 27.0 |

[a] Means of four determinations.
[b] Based on protein content of the dried crude gluten.

tized by each sucrose increment. The magnitude of the increase in soluble protein with each increase in sucrose concentration tended to be greater in the dispersions heated at 100°C. than in those held at 25°C.

The concentration of the protein was the most significant factor in determining the percent of protein peptized. In all cases the percent protein solubilized increased as the ratio of protein to solvent decreased. The lowest percent of protein extracted was that from the most concentrated dispersion in distilled water and was 7.24% at 25°C. and 4.88% at 100°C. The highest percent of protein peptized was from the most dilute protein dispersions and was 26.81% at 25°C. and 27.00% at 100°C.

Heating the gluten dispersion in a boiling-water bath for 1 hour resulted in a diminution in peptized protein at each protein and sucrose concentration, as compared with the same concentrations dispersed at room temperature. The increase in the soluble protein with each increase in sucrose in the heated dispersions indicates that sugar inhibits the heat coagulation of the gluten protein.

## Discussion

The importance of the peptization of gluten by sucrose in contributing to the tenderization of baked flour mixtures is questionable. Large quantities of liquid to flour (7:1) or gluten proteins (250, 150, and 50:1) were used in this investigation, whereas relatively small quantities of liquid are used in most sweet batters or doughs (<1:1). The amount of soluble proteins extracted from gluten dispersions has been shown to decrease as the ratio of liquid to gluten is decreased. In addition, the conditions used in extraction of soluble protein were severe in that the gluten was subjected to the shearing action of a Waring Blendor and the time of contact between the protein and the dispersing medium was approximately 24 hours, whereas a chemically leavened batter or dough receives more gentle handling and is usually baked within a short time following mixing.

It appears more likely, on the basis of these findings, that the tenderizing effect of sugar on baked products is due to a competition between sugar and the proteins for water, resulting in a lack of or reduction in gluten development. Additional work on the effect of sugar on the heat coagulation of wheat proteins is in progress.

The emphasis given the wheat proteins in this study does not preclude the importance of the function of the flour fractions other than protein as they may be affected by sucrose and as they may contribute to tenderization of baked products.

**Acknowledgment**

The authors are grateful to the Highspire Flour Mills Division of the Wheatena Corporation, Highspire, Pennsylvania, for the sample of flour.

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# SOME OBSERVATIONS ON THE CONSTITUTION OF WHEAT FLOUR LIPIDS ISOLATED FROM UNBLEACHED AND CHLORINE DIOXIDE-TREATED FLOURS[1]

K. A. Gilles, C. A. Anker, D. H. Wheeler, and J. S. Andrews

**ABSTRACT**

Portions of untreated patent flour were bleached with several levels of chlorine dioxide. The flours were extracted with pentane; the resultant lipids were concentrated *in vacuo* under reduced pressure at low temperature and were analyzed by ultraviolet and infrared spectrophotometric techniques. Contrary to the statements of opinion and speculation that have recently appeared in the literature, the treatment of patent flour with chlorine dioxide does not appear to alter appreciably the essential fatty acid content of the flour lipids.

The current high degree of interest in lipid nutrition studies has focused attention upon a number of hitherto unanswered questions. Among these are problems relating to the possible chemical changes that are effected by oxidizing agents used for flour bleaching to the nutritionally essential, polyethenoic fatty acids. Although opinions

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and speculations concerning this problem may be found in the literature (4,8,9), chemical data supporting these contentions are wanting.

It has been shown that the thiobarbituric acid value of flour lipids appeared to increase with bleaching. However, the quantitative extent of this effect varied markedly with the solvent used for extraction of the lipids and storage conditions employed; petroleum ether was the solvent least prone to effect autoxidation (5). Qualitative ultraviolet absorption studies have shown no very marked change in spectrum when the untreated flours were treated with agene or chlorine dioxide, although there was an increase in absorption in the region of 210–240 $m\mu$ in the case of the lipid from chlorine dioxide-treated flour (2).

An attempt to elucidate this facet of the problem gave rise to the following interesting series of experiments. Using the techniques of ultraviolet and infrared spectrophotometry, the authors analyzed lipids extracted from an unbleached and untreated patent hard wheat flour. To test the effect of treatment with chlorine dioxide, this flour was bleached with several levels of chlorine dioxide up to 1.0 g/cwt and the lipids, likewise, were analyzed.

## Materials and Methods

*Flour.* An unbleached hard wheat patent bread flour (protein 12.0%, ash 0.41% on a 14% moisture basis) was obtained for this work. The fat content of this flour was 1.34% by acid hydrolysis and 0.85% by pentane extraction.

*Bleaching.* The flour was bleached with chlorine dioxide according to the procedure of Parker and Fortmann (7). Levels of chlorine dioxide addition were 0, 0.05, 0.1, 0.5, and 1.0 g/cwt.

*Extraction of Lipid.* The flours were extracted with N-pentane, having a boiling range of 35–37°C., for 24 hours in a continuous, Soxhletlike extractor. The extraction was effected under conditions whereby the temperature of the extracting liquid was maintained at approximately 15°C.

*Concentration of Lipid Extract.* After filtration, solvent was removed from the flour extract by vacuum distillation. Final traces of solvent were removed from the extracted lipids under vacuum by means of a Rinco Rotating Film Concentrator fitted with an auxiliary condenser.

*Spectrophotometric Analyses.* The ultraviolet, spectrophotometric analyses of polyunsaturated acids were conducted according to the AOCS tentative method Cd 7-48 as revised April 1956 (1).

The infrared spectrophotometric analyses were conducted in a

Beckman Model IR-3, using the scanning technique in the range 2–15 $\mu$. Carbon disulfide solutions were used in the range 15–7.8 $\mu$ and carbon tetrachloride solutions in the range 7.8–2 $\mu$. Solutions of 6% concentration were employed in 0.1-mm. cells throughout the entire study.

*Miscellaneous Analyses.* All analyses which furnished supplemental information, i.e., iodine value, etc., were conducted according to recognized AOCS methods (1).

## Results and Discussion

The lipid contents of the flours which were subjected to different bleaching treatments are given in the table below. Approximately the

*Data Pertaining to the Thoroughness of Extraction of Flour Lipids*

| *Flour treatment* | *Fat extracted by pentane* |
|---|---|
| *g $ClO_2$/cwt* | % |
| 0.0 | 0.85 |
| 0.05 | 0.84 |
| 0.10 | 0.84 |
| 0.50 | 0.85 |
| 1.0 | 0.85 |

same amount of fat (0.85%) was extracted with N-pentane from each flour regardless of the previous bleaching treatment.

Prior to subjecting the lipids to spectrophotometric analysis, iodine values were determined for the series as indicated in the table below. No significant decrease in iodine value was observed with increased chlorine-dioxide treatment.

*Iodine Values of Chlorine Dioxide-Treated Flour Lipids*

| *Flour treatment* | *Iodine value* |
|---|---|
| *g $ClO_2$/cwt* | |
| 0.0 | 114.1 |
| 0.05 | 114.8 |
| 0.10 | 115.4 |
| 0.50 | 114.0 |
| 1.0 | 113.4 |

When lipids are subjected to ultraviolet spectrophotometric analysis (1), the linoleic (9,12-octadecadienoic acid) and linolenic acids (9, 12,15-octadecatrienoic acid) are determined directly. Oleic acid (9-octadecenoic acid) is calculated from iodine value and the determined content of linoleic and linolenic acids; so the errors of both analyses are combined in the percent of oleic acid calculated. Since saturated fatty acids are determined by difference, errors of the three previous determinations are combined in this value.

Ultraviolet analysis of the wheat flour lipids prior to alkaline isomerization did not show more than a trace of naturally occurring conjugated diene and triene. In the analysis of wheat flour oils presented in Table I, there is an apparent slight increase in the linoleic acid content and an erratic trend toward a slightly increased content of linolenic acid. However, the differences are no greater than found in collaborative tests on identical samples of oils (6). The oleic acid content appeared to decrease slightly and the saturated acid content appeared to increase slightly, in an erratic manner, with increased chlorine-dioxide treatment. The slight variations in analyses of the various samples showed no very consistent trends with respect to increasing chlorine-dioxide treatment. These observations confirm the independent ultraviolet analyses of Fisher *et al.* (3), who concluded that there would appear to be little significant change in the supply of essential fatty acids in chlorine dioxide-treated as compared with untreated flour.

TABLE I

LIPID ANALYSIS OF CHLORINE DIOXIDE-TREATED FLOURS
(Ultraviolet technique)

| TYPE OF FATTY ACID | PERCENT FATTY ACID IN FLOUR TREATED WITH CHLORINE DIOXIDE (G./CWT.) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.05 | 0.10 | 0.50 | 1.0 |
| Linoleic acid | 43.9 | 47.7 | 47.7 | 48.1 | 48.1 |
| Linolenic acid | 3.0 | 3.5 | 3.3 | 3.1 | 3.3 |
| Oleic acid | 34.9 | 26.7 | 27.9 | 26.2 | 24.8 |
| Saturated acids | 18.2 | 22.3 | 21.1 | 22.6 | 23.8 |

As an independent check, these lipids were subjected to infrared spectrophotometric analysis in the range of 2 to 15 $\mu$. The five infrared spectra were, for all practical purposes, indentical and confirmed the ultraviolet analyses. Typical spectra appear in Figs. 1 and 2 which show the spectral absorption curves obtained from lipids extracted from flours which were bleached with 0 and 1.0 g. $ClO_2$/per cwt.

In the isolated trans band region (10.34 $\mu$) all samples were very much alike. In the cis (3.31 $\mu$) and conjugated trans (10.12 $\mu$) carbon-to-carbon double-bond regions, only very small differences were observed. Differences in the amount of conjugated trans, trans double-bond material present in the various samples did not exceed 4% of the total amount of material present. These minor differences could not be correlated with the amount of chlorine dioxide used.

These experiments lead to the conclusion that, contrary to recent speculations, treatment of bread flour with chlorine dioxide does not

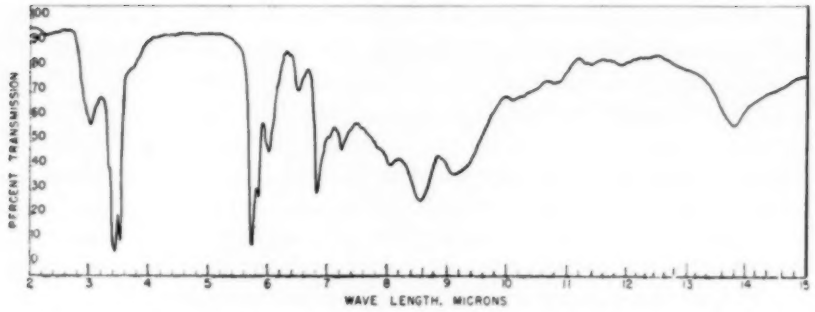


Fig. 1. Infrared spectrum of wheat flour lipids extracted with N-pentane from unbleached hard wheat flour.

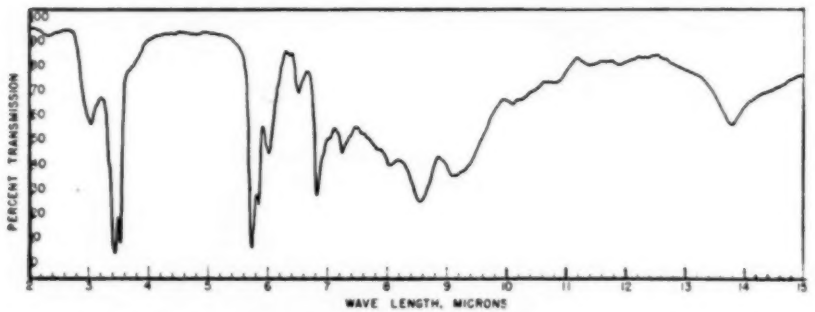


Fig. 2. Infrared spectrum of wheat flour lipids extracted with N-pentane from hard wheat flour bleached with 1.0 g. $ClO_2$/per cwt.

decrease the amount of the nutritionally important polyethenoic fatty acids that are normally present in the wheat flour lipids.

### Acknowledgments

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# A METHOD FOR DETERMINATION OF BRAN IN FLOUR[1]

W. L. Deatherage and Majel M. MacMasters


## ABSTRACT

A method has been developed for the direct determination of approximately one-third of the bran in flour. The other constituents of the flour are largely removed by solution or dispersion with alkali, followed by filtration. About two-thirds of the bran is also dissolved. The bran particles remaining on the filter are transferred to a graduated tube, recovered in a centrifuge under controlled conditions and the volume measured. The bran particles divide, on centrifuging, into two layers. The upper layer is white or colorless and the lower is brown. The weights of the two layers were determined separately. The volume of the particles recovered and their dry weight are nearly linearly related. Volume or weight of either total particles recovered or of brown particles is not clearly related to the ash content of the flour. There is some relationship between volume or weight of either total particles or of brown particles with the Kent-Jones & Martin Color Grader value, but the relationship is not linear. Photomicrographs show that in reality most of the bran is white or colorless and that often it is only a relatively small portion of seed coat or pigment strand which causes a whole particle to appear brown.


Cereal chemists recognize that the ash content is inadequate as an indication of the bran content of flour. The endosperm of some wheats is high in ash constituents and therefore yields flours of high ash content when few bran particles are present. Miller and Johnson (5) have discussed the disadvantages of current methods for the determination of bran in flour as part of the evaluation of flour quality. Larkin *et al.* (4) reviewed most of the previously proposed methods for bran determination and suggested a method for staining bran and germ particles with Crystal Violet for rapid microscopic identification. Their method was later found to be applicable to some flours but not to others.

The need for a rapid method for bran determination, suitable for use in the control laboratory, was recognized by the American Association of Cereal Chemists when a subcommittee was set up in 1952 to study the Crystal Violet method. The subcommittee later evolved into the Bran-in-Flour Committee, designed to test new and modified methods. It was early recognized that existing methods were inadequate for the accurate determination of the bran content of flour. In an attempt to fulfill this evident need, the present study was undertaken to develop a new method for the determination of bran in flour.

[1] Manuscript received January 27, 1958. Contribution from the Northern Utilization Research and Development Division, Peoria, Illinois, one of the Divisions of the Agricultural Research Service, U. S. Department of Agriculture. Presented in part at the 41st annual meeting, New York, May, 1956.

## Materials and Methods

*Equipment and Supplies.* The equipment and supplies required for carrying out the determination of bran in flour by the new method are listed.

Pyrex serum bottle, with rubber stopper, 4-liter capacity
Graduated cylinder, 2-liter capacity
Graduated cylinder, 100-ml. capacity
Suction flask, 4-liter capacity
Büchner funnel, 1,200-ml., stainless steel, Micro Metallic,[2] with prealloyed stainless-steel powder porous disk, E-medium porosity, average pore size 35$\mu$.
Centrifuge tubes, 125-ml., pear-shaped, Pyrex glass, with straight stem graduated from 0 to 1.5 ml. in 0.1-ml. divisions (as specified for ASTM test D-96-47T)
Centrifuge with head, trunnion carrier, and cushions for holding the specified centrifuge tubes
Rubber policeman
Sodium hydroxide pellets, 97% assay
Isopropanol, 99%

*Samples Studied.* The samples studied included a group 15 flours furnished by members of the Bran-in-Flour Committee of the American Association of Cereal Chemists and 23 mill-stream samples from one variety of hard red winter wheat milled on the large experimental mill at Kansas State College, Manhattan, Kansas. In preliminary studies, bran separated from Thatcher variety wheat was used. Commercial bakery flour was used for preparing photomicrographs and in some exploratory studies.

*Methods.* The method developed for the separation and determination of bran in flour is as follows:

Twenty-five grams of flour (dry basis) are weighed to the nearest 0.1 g. and put into a clean, dry, 4-liter serum bottle. One hundred milliliters of isopropanol are added and the suspension is swirled gently until all lumps disappear from the flour. Then 2,500 ml. distilled water and 12.5 g. of sodium hydroxide pellets are added. The bottle is shaken for 0.5 minute, or until the sodium hydroxide dissolves, and as often thereafter as is required to keep the flour from settling out. After 1 hour, the dispersion is filtered through the E-medium porosity Micro Metallic filter, with suction. The serum bottle is washed twice with 100-ml. portions of distilled water and the washings are passed through

[2] The mention in this article of commercial products does not constitute an endorsement of such firms or products by the U. S. Department of Agriculture.

the filter. The fiber particles remaining on the filter disk are washed three times with 50-ml. portions of distilled water and then backwashed off the filter into the pear-shaped centrifuge tube. This is accomplished by pouring 25 ml. of distilled water onto the disk, connecting the stem of the filter to a compressed-air line and, by applying gentle pressure, bubbling air back through the filter. With the backpressure and the aid of a rubber policeman, the particles are worked loose from the disk and suspended in the water. The backwashing is repeated three times to assure removal of all particles. The four suspensions obtained by backwashing are combined in the centrifuge tube and centrifuged at 628 × gravity for a predetermined time. The volume of particles is read in the stem of the tube. Centrifuging beyond 5 minutes does not appear to reduce volume of recovered fiber particles. For determination of bran in flour, volume of total particles recovered is read. Duplicate determination should agree within 0.1 ml.

The filter disk is cleaned after being used twice. The disk is barely covered with dichromate cleaning solution. After a few minutes, suction is applied to draw the cleaning solution through the disk into the empty filter flask; finally, a large volume of distilled water is drawn through the disk to remove the remainder of the cleaning solution.

Two sets of duplicate determinations were made on all samples for which results are reported. First, the total volume of particles recovered was read and the weight of total particles was determined by transferring all of the sediment to a fritted-glass crucible in which it was filtered, dried, and weighed. Second, readings were made of volume of total particles, of brown particles, of the part of the diffuse layer more brown than white, and of the part of the diffuse layer more white than brown. (An arbitrarily chosen 1-hour centrifuging time was used throughout this second series of determinations only to ensure a better separation, for gravimetric analysis, of the brown particles from the mill-stream samples.) The brown particles alone were then transferred to a fritted-glass crucible, filtered, dried, and weighed.

Some of the mill streams that were tested contained so much bran that it more than filled the graduated stem of the centrifuge tube when the usual sample size was used. The sample size was in these cases decreased and the results calculated to the 25-g. basis.

Determinations of moisture and of ash were made by AACC methods (1).

The mill streams were tested on the Kent-Jones & Martin Color Grader (3) at Kansas State College.

In efforts to improve definition of separation of particles recovered, several different solvents, dyes, and electrolytes were added individu-

ally to alcohol-dried particles and the mixtures were centrifuged.

The presence of seed coat was confirmed by treating recovered particles with 70% sulfuric acid for 5 minutes to dissolve all other parts.

To test for differences in densities among recovered particles, alcohol-dried particles were suspended in carbon tetrachloride in a tall, narrow glass tube, then cyclohexane was added in small increments to vary the density.

## Results and Discussion

There appear to be two distinct fiber fractions recovered from wheat flour that differ from each other in color. The first is white or

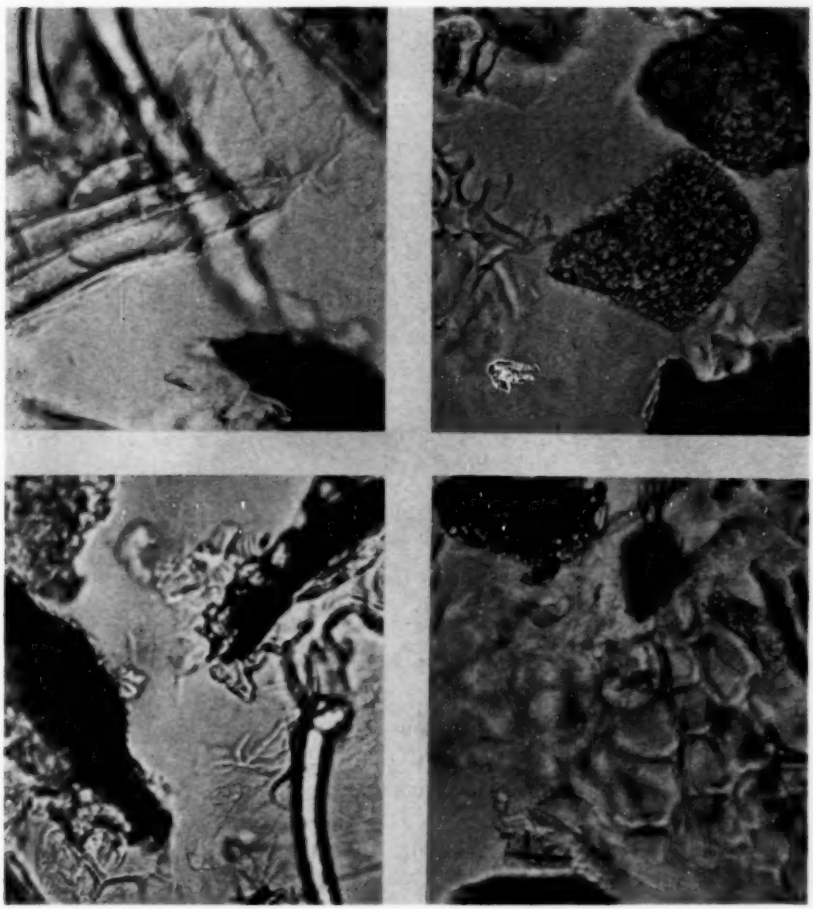

Fig. 1. Upper: Particles from brown layer. Lower left: Particles from brown layer. Lower right: Particles from diffuse layer. Magnification 170×.

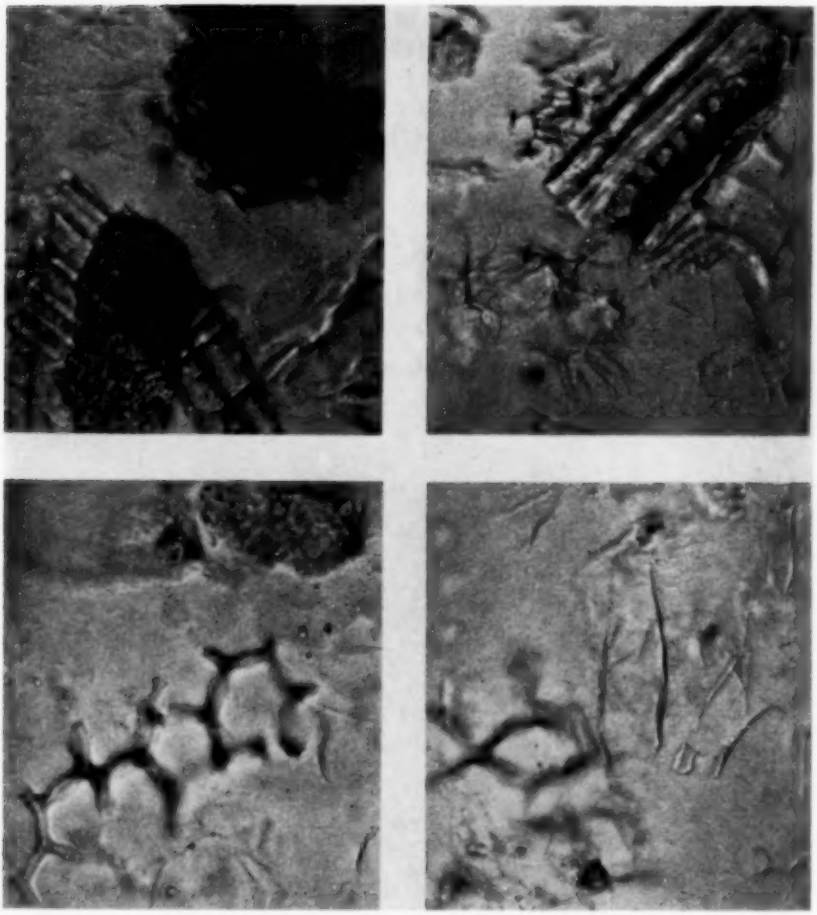

Fig. 2. Upper: Particles from diffuse layer. Lower: Particles from white layer. Magnification 170×.

nearly white. The second is brown. Between the two layers formed by these fractions upon centrifuging, there lies a diffuse layer composed of parts of both main fractions, but which is chiefly white. All three layers contain bran particles (Figs. 1 and 2). Color is determined by the presence and amount of seed coat and pigment strand in a given particle (2). The brown particles contain enough seed coat and/or pigment strand to give color to all, but the actual amount of these structures in a given particle may be relatively small. White particles contain little, if any, seed coat and pigment strand. These particles are composed largely of cell walls from the aleurone layer and starchy

endosperm. During application of the procedure, readings were taken of the volume of each of the layers.

Preliminary studies showed that the method recovered approximately one-third of the finely ground flake bran subjected to the treatment (Table I). Apparently some of the chemical constituents of the

TABLE I

RECOVERY OF BRAN SUBJECTED TO THE NEW METHOD

| DRY WEIGHT OF BRAN | VOLUME OF PARTICLES RECOVERED | WEIGHT OF PARTICLES RECOVERED | RECOVERY |
|---|---|---|---|
| *mg* | *ml* | *mg* | % |
| 50 | 0.20 | 14.5 | 29.0 |
| 100 | 0.40 | 30.2 | 30.2 |
| 150 | 0.60 | 47.4 | 31.6 |
| 200 | 0.80 | 65.8 | 32.9 |
| 250 | 1.00 | 83.2 | 33.2 |

bran were dissolved by the alkaline treatment, thus lowering the weight of the recovered particles. Possibly, too, some particles entered the filter disk and failed to be removed by the backwashing. There appeared to be no loss of particles through the filter. The filtrate was always free of visible particles, for no particles were recovered when it was passed through a finer filter. Few white particles were obtained when bran alone was subjected to the treatment.

When known amounts of finely ground bran were added to a commercial bakery flour, about one-third of the added bran was recovered (Table II). The average increases in volume and in weight were close to those which were obtained when equivalent amounts of the bran were processed without the presence of flour.

TABLE II

RECOVERY OF BRAN ADDED TO 25 GRAMS OF COMMERCIAL BAKERY FLOUR

| BRAN ADDED | VOLUME OF PARTICLES RECOVERED | INCREASE OVER BLANK | WEIGHT OF PARTICLES RECOVERED | INCREASE OVER BLANK | RECOVERY |
|---|---|---|---|---|---|
| *mg* | *ml* | *ml* | *mg* | *mg* | % |
| 0 | 1.00 | | 47.9 | | |
| | 1.05 Av. 1.02 | ... | 48.0 Av. 47.9 | ... | ... |
| 50 | 1.18 | | 60.4 | | |
| | 1.20 | | 65.0 | | |
| | 1.10 | | 64.1 | | |
| | 1.15 Av. 1.16 | 0.14 | 67.0 Av. 64.1 | 16.2 | 32.4 |
| 100 | 1.20 | | 81.5 | | |
| | 1.30 | | 85.5 | | |
| | 1.25 | | 77.2 | | |
| | 1.30 Av. 1.26 | 0.24 | 83.1 Av. 81.8 | 33.9 | 33.9 |
| 150 | 1.45 | | 98.5 | | |
| | 1.50 | | 97.6 | | |
| | 1.35 | | 94.6 | | |
| | 1.40 Av. 1.42 | 0.40 | 99.9 Av. 97.6 | 49.7 | 33.1 |

## TABLE III

DATA ON FIBER PARTICLES RECOVERED AND ASH IN FLOURS ARRANGED IN ORDER OF INCREASING WEIGHT OF BROWN PARTICLES (25-GRAM SAMPLE)

| Flour | Centrifuged Volume | | | | Weight (d.b.) | | Ash (d.b.) |
|---|---|---|---|---|---|---|---|
| | Total Particles | Brown Particles | Diffuse Layer, Brown | Diffuse Layer, White | Total Particles | Brown Particles | |
| | *ml* | *ml* | *ml* | *ml* | *mg* | *mg* | % |
| Spring wheat, Willet var. | 0.55 | 0.30 | 0.10 | 0.15 | 17.1 | 13.8 | 0.37 |
| Spring wheat, Selkirk var. | 0.55 | 0.30 | 0.10 | 0.15 | 19.1 | 15.1 | 0.43 |
| Spring wheat, Lee var. | 0.50 | 0.30 | 0.10 | 0.10 | 21.5 | 15.1 | 0.43 |
| Minnesota A | 0.50 | 0.30 | 0.10 | 0.10 | 21.6 | 16.4 | 0.41 |
| Northwest B | 0.80 | 0.40 | 0.10 | 0.30 | 27.1 | 19.6 | 0.37 |
| Commercial soft red winter | 0.70 | 0.30 | 0.20 | 0.20 | 27.5 | 19.3 | 0.40 |
| Northwest A | 0.70 | 0.40 | 0.20 | 0.10 | 35.0 | 25.0 | 0.51 |
| Family type | 0.85 | 0.40 | 0.20 | 0.25 | 36.1 | 21.8 | 0.41 |
| Northwest C | 0.90 | 0.50 | 0.20 | 0.20 | 39.1 | 28.4 | 0.45 |
| Bakery | 0.95 | 0.50 | 0.20 | 0.25 | 40.4 | 24.7 | 0.49 |
| Exptl. milled, soft wheat mixture | 1.10 | 0.50 | 0.20 | 0.40 | 41.4 | 32.0 | 0.43 |
| Commercial bakery | 1.10 | 0.70 | 0.20 | 0.20 | 46.0 | 35.7 | 0.50 |
| Minnesota B | 1.00 | 0.60 | 0.30 | 0.10 | 52.6 | 35.8 | 0.50 |
| Cookie | 1.40 | 0.60 | 0.20 | 0.60 | 66.8 | 42.8 | 0.39 |
| Exptl. milled, Trumbull var. | 1.50 | 0.80 | 0.20 | 0.50 | 69.1 | 52.1 | 0.48 |

## TABLE IV

DATA ON FIBER PARTICLES RECOVERED, ASH CONTENT, AND COLOR GRADER VALUE OF MILL STREAMS FROM A HARD RED WINTER WHEAT

| Mill Stream | Centrifuged Volume | | | | Weight (d.b.) | | Ash (d.b.) | Color Grader Value |
|---|---|---|---|---|---|---|---|---|
| | Total Particles | Brown Particles | Diffuse Layer, Brown | Diffuse Layer, White | Total Particles | Brown Particles | | |
| | *ml* | *ml* | *ml* | *ml* | *mg* | *mg* | % | |
| 1st Mid. top | 0.30 | 0.10 | 0.10 | 0.10 | 9.0 | 4.2 | 0.46 | 2.5 |
| 1st Mid. bottom | 0.60 | 0.30 | 0.10 | 0.20 | 17.7 | 10.6 | 0.47 | 2.0 |
| 2nd Mid. top | 0.55 | 0.30 | 0.10 | 0.15 | 20.2 | 13.1 | 0.42 | 1.6 |
| 2nd Mid. bottom | 0.60 | 0.30 | 0.10 | 0.20 | 21.8 | 12.9 | 0.44 | 1.5 |
| 3rd Mid. top | 0.60 | 0.30 | 0.10 | 0.20 | 23.5 | 13.9 | 0.46 | 2.3 |
| 3rd Mid. bottom | 0.70 | 0.40 | 0.20 | 0.10 | 29.2 | 19.0 | 0.45 | 2.4 |
| 4th Mid. top | 0.40 | 0.20 | 0.10 | 0.10 | 16.2 | 9.0 | 0.45 | 2.2 |
| 4th Mid. bottom | 1.30 | 0.50 | 0.20 | 0.60 | 55.5 | 30.5 | 0.40 | 2.4 |
| 5th Mid. | 0.95 | 0.60 | 0.20 | 0.15 | 39.8 | 29.8 | 0.42 | 2.3 |
| Sizings [a] | 1.85 | 0.50 | 0.25 | 1.10 | 64.2 | 28.0 | 0.47 | 2.5 |
| 1st Break | 0.85 | 0.60 | 0.10 | 0.15 | 47.3 | 40.3 | 0.52 | 4.0 |
| 2nd Break | 0.90 | 0.70 | 0.10 | 0.10 | 60.3 | 57.0 | 0.62 | 5.4 |
| 3rd Break | 0.70 | 0.60 | 0.10 | 0.80 | 50.9 | 48.1 | 0.53 | 5.0 |
| 4th Break | 1.10 | 1.00 | 0.10 | 0.00 | 72.4 | 70.7 | 0.73 | 7.3 |
| 5th Break [b] | 3.00 | 2.50 | 0.50 | 0.00 | 169.0 | 163.5 | 0.89 | 9.0 |
| 1st Tailings | 0.60 | 0.50 | 0.10 | 0.00 | 33.0 | 27.4 | 0.58 | 4.5 |
| 2nd Tailings [b] | 1.75 | 1.00 | 0.50 | 0.25 | 70.5 | 50.7 | 0.73 | 5.4 |
| 1st, 2nd, and 3rd Break redust | 1.40 | 1.10 | 0.10 | 0.20 | 92.7 | 83.0 | 0.51 | 4.3 |
| Bran and shorts, duster top | 1.00 | 0.90 | 0.10 | 0.00 | 64.2 | 55.6 | 0.66 | 5.8 |
| Bran and shorts, [b] duster bottom | 4.50 | 3.50 | 0.50 | 0.50 | 236.7 | 197.2 | 0.64 | 7.4 |
| 1st Low-grade [b] Top | 6.50 | 4.00 | 1.25 | 1.25 | 280.7 | 215.7 | 0.69 | 7.1 |
| 1st Low-grade [b] Bottom | 3.00 | 1.50 | 1.00 | 0.50 | 120.7 | 82.0 | 0.64 | 4.7 |
| 2nd Low-grade [b] Top | 6.25 | 4.00 | 1.00 | 1.25 | 269.0 | 197.0 | 0.90 | 8.0 |

[a] 20-Gram sample used, calculated to 25-g. sample.
[b] 5-Gram sample used, calculated to 25-g. sample.

The experimental data obtained by application of the method to flours and to mill streams are summarized in Tables III and IV. The agreement between duplicate determinations of either total particles or brown particles of the samples was within 0.1 ml. of volume and within 0.01 g. of weight after drying. These errors were on the basis of the weight of sample used; in cases where small sample weights were used and results calculated to the 25-g. basis, the final errors were somewhat larger.

There are some instances in which comparison of two or more samples shows that increase in volume of total particles from sample to sample is unaccompanied by a proportional increase in weight of the particles. A similar condition is found when the volumes and weights of brown particles only are compared. The cause may be a

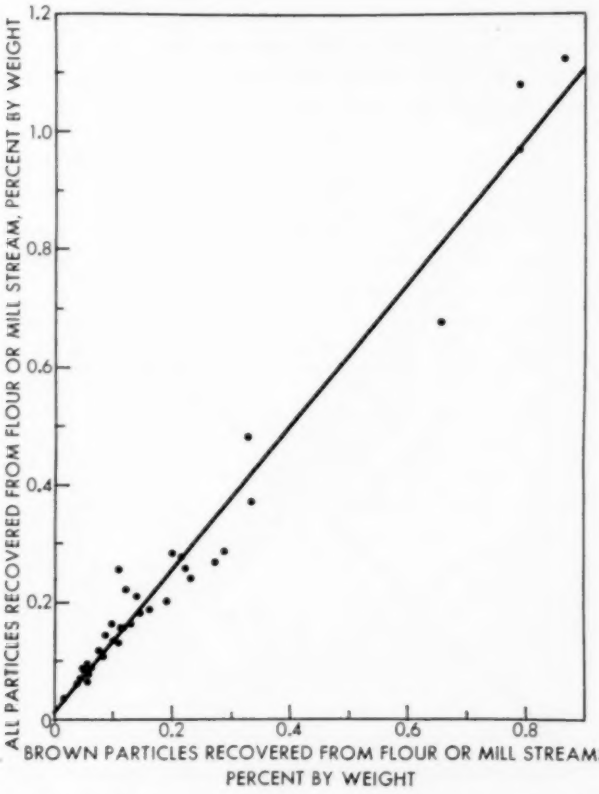


Fig. 3. Relationship between the percent by weight of total particles recovered and the percent by weight of brown particles in the flour and mill-stream samples.

difference in the size and shape of the particles (which would affect packing in the certrifuge tube).

The relationship between percentage of total particles and percentage of brown particles, calculated on a weight basis, was relatively constant (Fig. 3).

The volume of brown particles, read from the centrifuge tube, was proportional to the percentage of the particles, by weight, in the sample (Fig. 4). The data from samples with high bran contents had to be calculated from the 5-g. sample to the 25-g. basis; errors were consequently magnified fivefold. On the 5-g. basis, the points above 3-ml. volume would lie as close to the line as do the points below that level.

The well-known lack of relationship between bran content and ash content of flour is graphically illustrated in Fig. 5. Here the percentage of brown particles in the flour (on a weight basis) has been plotted against the ash content. A similar diagram was obtained by plotting weight percentage of total particles against the ash content.

Some relationship was found between the ash content and the Color Grader values of the mill stream samples (Fig. 6).

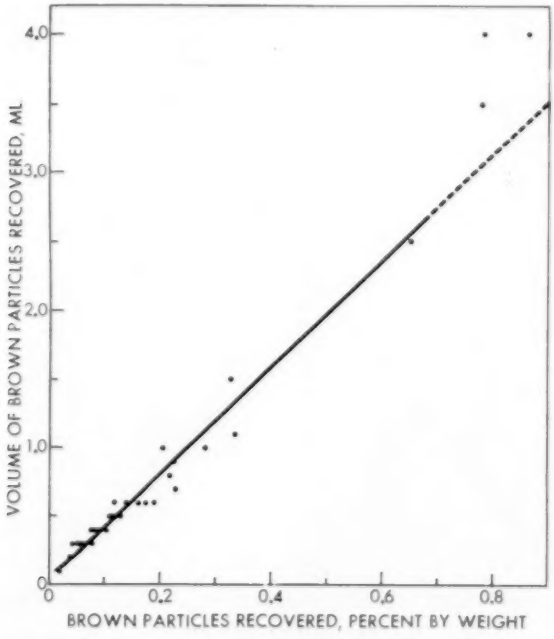


Fig. 4. Relationship between the volume of brown particles and the percent by weight of brown particles in the flour and mill-stream samples.

Comparison of the percentage of brown particles in the mill-stream samples with the Color Grader values of the samples (Fig. 7) indicates that the Kent-Jones & Martin Color Grader gave a value which was not directly proportional to the amount of brown particles present. When the same data were plotted on semilogarithmic paper, the

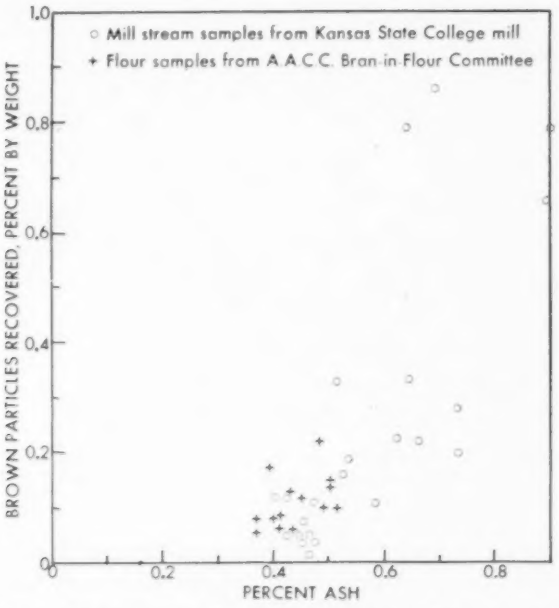


Fig. 5. Relationship between the percent by weight of brown particles and the percent ash of the flour samples. ○ — mill-stream samples from Kansas State College mill. + — flour samples from AACC Bran-in-Flour Committee.

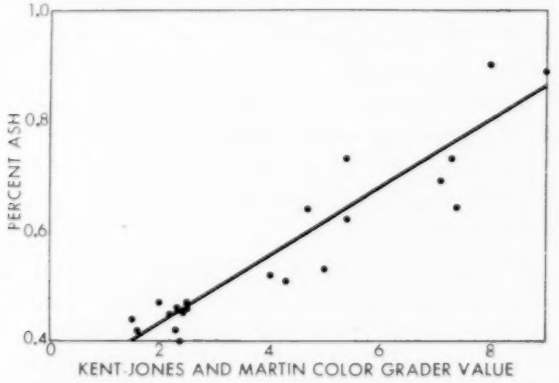


Fig. 6. Relationship between the percent ash and the Kent-Jones & Martin Color Grader value of the mill-stream samples from Kansas State College mill.

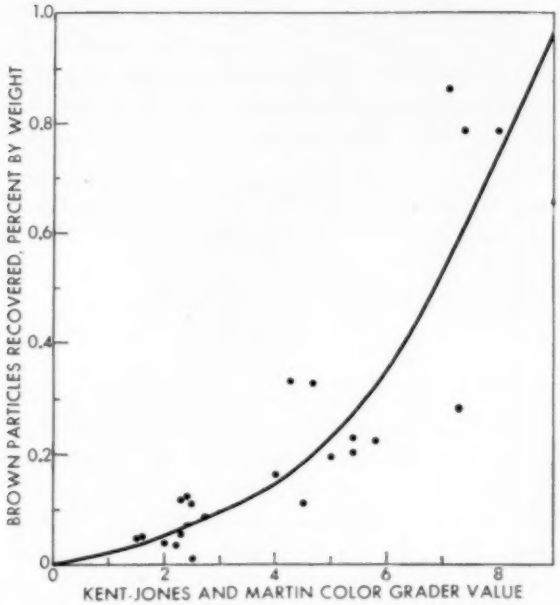


Fig. 7. Relationship between the percent by weight of brown particles and the Kent-Jones & Martin Color Grader value of the mill-stream samples from Kansas State College mill.

lack of linear correlation was confirmed. A similar, but not exactly duplicate, curve was obtained when the percentage of total particles recovered from the samples was plotted against the Color Grader value. The Color Grader appeared, however, to give a better indication of brown particle content than was obtained by determination of the ash content (compare Figs. 5 and 7). Further work on a wide variety of flour samples will be needed before the value of the Color Grader for determination of bran content of flour can be firmly established.

All efforts to improve separation between brown and white particles recovered in centrifuge tubes failed.

The particles suspended in carbon tetrachloride and cyclohexane mixtures of various densities failed to separate into sharply defined brown and white layers.

From present data, the volume reading of the total particles recovered after centrifuging appears to be the best criterion available for estimation of bran content of wheat flour. Collaborative testing of the method will be required before its true value to the miller and baker can be established. This work is contemplated in the near future.

Exploratory tests with single samples of rye, corn, and oat flours suggest that this method for the determination of bran in wheat flour might successfully be adapted for use on other flours.

**Acknowledgment**

The authors gratefully acknowledge the aid of those who furnished flours for testing: M. A. Barmore, L. S. Cuendet, R. H. Harris, H. K. Heizer, and T. H. McCormick; of J. A. Shellenberger who supplied the mill-stream samples; of B. S. Miller for determining Kent-Jones & Martin Color values; of J. E. Hubbard who supplied the bran; of I. M. Cull for developing the pictures; and of M. J. Wolf for taking the photomicrographs.

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# NOTE ON A RAPID METHOD TO SCREEN CORN SAMPLES FOR APPROXIMATE AMYLOSE CONTENT IN THE STARCH[1]

R. R. Savage, W. L. Deatherage, Majel M. MacMasters, and F. R. Senti[2]

For the past six years the Northern Utilization Research and Development Division has been collaborating, under memoranda of understanding, with the Bear Hybrid Corn Co., Inc., Decatur, Ill., and with Production Research of the United States Department of Agriculture's Agricultural Research Service (represented by Dr. M. S. Zuber, Crops Research Division, stationed at the Missouri Agricultural Experiment Station), in the development of field-type corn containing starch with a high amylose content. Published results indicate that promising progress was made (1,2). More recently, corn with 80% amylose in the starch was developed by Dr. Zuber.[3]

The analytical chemical analysis of corn samples provided by these breeders was done by the Northern Division. During the first 5 years of cooperative work, only one or two thousand analyses for amylose were required annually. Because of increasing emphasis on the project, over 7,000 samples were analyzed during the first 9 months of 1957, with work building up to analyses of 1,000 samples per month.

Normally, such a large volume would require a staff of about 20 workers. Endosperm from each individual corn sample has to be dissected out and ground; oil is extracted from it by refluxing with 85% methanol. Then it is necessary to wash, dry, regrind, and to weigh samples for analyses, determine moisture and Kjeldahl nitrogen (to give the basis for calculation of weight of starch in the sample taken for titration), make potentiometric titration with iodine for determining amylose content in the starch, and calculate the results. Obviously, a more rapid procedure was desirable.

A relatively rapid screening method was developed for eliminating from further consideration samples with starch of less than a chosen amylose content. Basis of the method is the degree of gelatinization of starch brought about by a definite alkali concentration and controlled heating. High-amylose starch resists gelatinization in comparison with ordinary starch.

[1] Manuscript received January 24, 1958.

[2] Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

[3] U. S. Department of Agriculture. "High-amylose corn may find wide industrial use." Press Release 2701-57 (Sept. 4, 1957).

The method consists of soaking a 3- to 7-g. sample of kernels in distilled water for 30 minutes, separating the endosperm by hand, air-drying the endosperm, grinding it to pass a 40-mesh screen, placing 1 g. of the dry ground material in a 75-ml. test tube, adding 25 ml. of 0.111*N* potassium hydroxide solution, heating the suspension in a circulating bath at 75°C. until that temperature is reached by the suspension, continuing heating for 3 to 4 minutes with stirring, removing the suspension from the bath, neutralizing with 1*N* hydrochloric acid, and microscopically examining one drop of the suspension mixed with one drop of 0.05% aqueous solution of Congo Red. It is convenient to use a polarizing microscope with a 20X ocular and 20X objective. Congo Red is added to help the observer; all gelatinized granules or portions of granules stain with Congo Red, whereas granules or portions of granules that still show birefringence remain unstained. Either birefringence or lack of staining with Congo Red may be used alone to identify ungelatinized starch.

The method was first roughly standardized by applying it to samples with starch of known amylose content. Usually a sample with starch having a 50% amylose content has one to a few ungelatinized granules when treated as indicated; at 50 to 60% amylose content, several granules are still ungelatinized; many granules remain ungelatinized when the amylose content of the starch is above 60%. It is also possible to adjust the concentration of potassium hydroxide and the temperature to obtain similar results at higher amylose levels.

Comparison of amylose content estimated by the rapid screening method with actual amylose content of the starch as determined by titration is shown for 480 samples in Table I. As might be expected, accuracy of classification decreased as amylose content of the samples approached the limits of the classes. For example, less than 1% of the samples having amylose contents in the range 0 to 44% were placed

TABLE I

COMPARISON OF AMYLOSE ESTIMATED BY SCREENING WITH AMYLOSE FOUND BY TITRATION

| Amylose by Titration | | Amylose by Screening | | |
|---|---|---|---|---|
| Amylose Content | Number of Samples | Number of samples in range | | |
| | | <50% | 50-60% | >60% |
| % | | | | |
| 65–71 | 30 | 0 | 7 | 23 |
| 60–64 | 68 | 3 | 29 | 36 |
| 55–59 | 140 | 12 | 79 | 49 |
| 50–54 | 87 | 24 | 49 | 14 |
| 45–49 | 26 | 20 | 6 | 0 |
| 0–44 | 129 | 128 | 1 | 0 |

in the 50–60% class by the screening method. Nearly one-third of the samples having an amylose content in the 45–49% range, however, were classified in the 50–60% range. Similarly, about one-third of the samples in the 65–71% range were estimated to contain 50–60% amylose.

The limits to be set in using the screening method will depend on what amylose range is wanted for further study. If samples having amylose contents of 60% and above are desired, 59 out of 98 samples from the group analyzed in Table I would be retained by setting the lower limit at > 60% in the screening. This screening limit also would retain 63 samples, having actual amylose contents below the desired range, 60% and above. Nearly all samples having amylose contents in this range could be retained by setting the lower screening limit at 50% amylose. In the group described in Table I, only three of the 98 samples having amylose contents above 60% would be discarded. However, an additional 135 samples with amylose contents below 60% would be retained. Nevertheless, 174 samples, or about 35% of the total, would be eliminated from further consideration.

In practical use, this method has eliminated from further consideration some 25 to 45% of samples in various series. This elimination has materially reduced the number of workers required and has facilitated rapid return of analytical results to the plant breeders. The method can be applied, for rough screening, to endosperm cut from the top of individual kernels. The remainder of the kernel could subsequently be planted for increase, if desired.

Another feature of this screening test is that it is also applicable to starch which has been separated from other endosperm constituents. The starch is immediately suspended in the potassium hydroxide solution and heated. With proper standardization, the method could be applied to other cereal grains, and, indeed, to other starches and plant parts containing starch.

**Acknowledgment**

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# A METHOD FOR THE DETERMINATION OF THE SURFACE AREA OF WHEAT KERNELS[1]

JOE R. HART[2] AND LOUIS FEINSTEIN[2]

**ABSTRACT**

The surface area of wheat kernels may be determined by coating them with silver and determining the surface area of the silver by measuring the extent to which it catalyzes the decomposition of a standard solution of hydrogen peroxide. Determinations were made on samples of wheat separated into different kernel-size groups by sieving. The mean surface area per wheat kernel was found to be 22, 27, 34, 42, and 48 sq. mm. for mean kernel weights of 0.0131, 0.0208, 0.0281, 0.0371, and 0.0453 g. respectively.

Methods which are often used for measuring the surface area of particles do not appear to lend themselves readily to such determinations on wheat kernels. Those methods involving the adsorption of gases (3,7) or the measurement of the permeability of the particles to fluids (4) are better suited for use with small particles where the ratio of area to mass is high. Furthermore, adsorption not only would take place on the external surface but would involve a diffusion to the inside of the kernels (1,8). It is believed that this would also be the case in the adsorption of a dye (9). If surface area is determined by the method of measuring the rate of imbibition (6), it must be assumed that the liquid is taken in uniformly over the entire particle surface. This will not be true, however, for kernels of wheat (2).

A method which does not involve any of these procedures was therefore chosen for investigation. A modification of the method used by Clark (5) in determining the specific surface of wood pulp was found useful for determining the surface area of wheat kernels and is described here. It involves the coating of the kernels with silver through the reduction of a solution of ammoniacal silver nitrate, and the determination of the silver surface area by measuring the extent to which the surface catalyzes the decomposition of a standard solution of hydrogen peroxide. An advantage of this method is the fact that the degree of decomposition of the hydrogen peroxide is determined by the silver surface area and is not affected by lack of uniformity in the thickness of the silver coating (9). The extent of decomposition of the hydrogen peroxide solution is a function of silver surface area, hydrogen peroxide concentration, time, and temperature. Since the decomposition of peroxide takes place only above a certain pH, a timed reaction

[1] Manuscript received January 6, 1957.

[2] Biological Sciences Branch, Agricultural Marketing Service, U. S. Department of Agriculture, Beltsville, Md.

can be obtained simply by adding sulfuric acid at the end of the time period selected.

## Experimental Procedure

*Coating of Kernels with Silver.* The silvering solution is prepared by adding dilute ammonium hydroxide solution very carefully to a 2% solution of silver nitrate until the color of the brown precipitate formed lightens and barely disappears. A weighed sample of wheat (300 kernels) is placed in 150 ml. of this solution which has been brought to boiling in a beaker. Boiling is continued until all the kernels turn a deep black color owing to plating out of the silver. This usually requires 3 to 5 minutes. The solution is then poured off and the kernels are washed thoroughly by means of several additions of distilled water, with stirring and decanting after each addition.

To determine whether swelling of kernels took place during the silvering process, owing to imbibition, the following test was carried out on different volumes of wheat kernels. A cream test bottle having a slender graduated neck was filled with liquid to the lower end of the neck and the volume level was read accurately. The kernels being tested were then placed into the liquid and the new level was read. The difference between the two readings gave the volume of the kernels added. After silvering, the volume of the kernels was again determined. Skellysolve F has been found to be a satisfactory liquid. There appeared to be no swelling taking place while the volume determinations were being made, and the attachment of air bubbles to the kernels, so troublesome when water is used as the liquid, was largely eliminated. The addition of water to kernels which had been previously immersed in Skellysolve F showed that their power to imbibe water had not been materially decreased. The results of the determinations are given in the table below. These results show an increase

*Effect of Silvering on the Volume of Wheat Kernels*

| *Sample* | *Volume of kernels before silvering* | *Volume after silvering* |
|---|---|---|
| | *cc* | *cc* |
| 1 | 4.59 | 4.78 |
| 2 | 5.12 | 5.34 |
| 3 | 4.06 | 4.21 |
| 4 | 3.96 | 4.13 |
| 5 | 5.02 | 5.21 |

in volume of approximately 4% during the silvering process. It is believed that this small increase would produce a much smaller increase in surface area, since its effect would be to smooth or round out the creased and irregular surface of the kernel.

*The Timed Reaction with Hydrogen Peroxide.* The reaction with

hydrogen peroxide is carried out in a titration flask having a mouth large enough to accommodate the shaft of a motor-driven stirrer, a thermometer, the tip of the buret from which standard permanganate solution is added, and a separatory funnel. The silver-covered sample (300 kernels), 100 ml. of distilled water, and 5 ml. of sodium borate buffer solution (25 g. hydrated sodium borate and 20 ml. *N* sodium hydroxide diluted to 500 ml.) are added to the flask and the stirrer motor started. A 10-ml. portion of a hydrogen peroxide solution, equivalent in these experiments to 6.20 ml. of 0.03*N* potassium permanganate, is pipetted into the separatory funnel. The peroxide solution is then delivered rapidly into the flask from the separatory funnel and a stop-watch started. At the end of 50 seconds, the temperature of the reaction is noted. Exactly at the end of 100 seconds, 15 ml. of 2*N* sulfuric acid solution are poured into the flask to stop the reaction. The amount of peroxide not decomposed is found by titrating the contents of the flask with 0.03*N* potassium permanganate solution to a faint pink end point. The area of the sample tested is determined by reference to a graph prepared by applying the identical procedure to spherical glass beads of the same size order as wheat kernels. In all of these experiments, it is necessary to use great care in keeping the apparatus scrupulously clean, because contamination may poison the silver catalyst.

*Preparation of the Graph.* The average diameter of 100 beads measured was 3.48 mm., with the range of diameters from 3.40 to 3.80 mm. Departure from sphericity was determined by measuring the diameters of each bead along several axes and taking the difference between the largest and smallest diameters. The largest difference found was 0.03 mm. All measurements were made with micrometer calipers. The surface area per bead was 38.06 sq. mm. Determinations of the surface area by the method given above were then made on groups of 200, 250, 300, 400, and 500 beads. The log of the number of milliliters of 0.03*N* potassium permanganate required to react with the hydrogen peroxide not decomposed by the silver surface in each determination was plotted against the total calculated area of the beads taken. This relationship is shown in Fig. 1.

## Results and Conclusion

Determinations of surface area were made on three different samples of wheat by this method. Each sample was first, however, separated into different kernel size groups by sieving. The area values obtained and other pertinent information are given in Table I. The smaller the kernel size is, the greater the surface area per g. In all of the de-

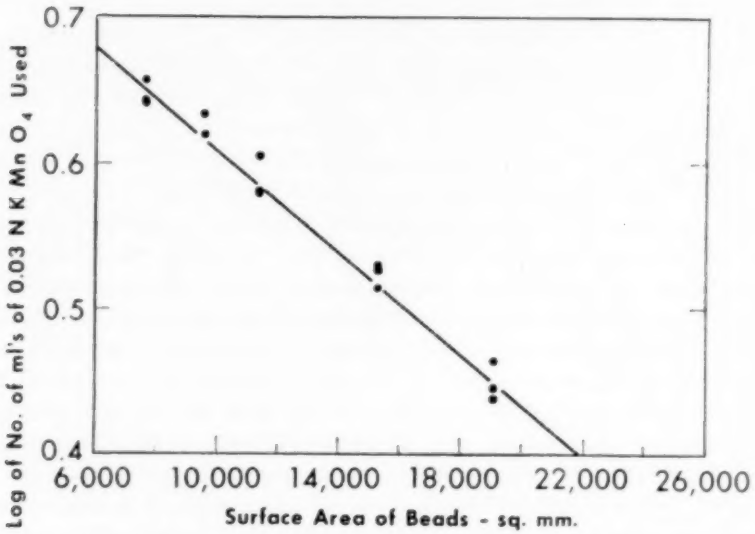


Fig. 1. Relation of silver surface area and hydrogen peroxide decomposed by the silver surface, as determined by potassium permanganate titration. (Source: U.S. Department of Agriculture, Agricultural Marketing Service.)

terminations reported, both on the bead samples and on the wheat samples, the temperature did not deviate from 25°C. by more than 2°C. When a temperature correction factor was calculated from the data, the correction was negligibly small in relation to the deviation of individual determinations from the graph. A small additional source of error lies in the fact that the concentration of the hydrogen perox-

TABLE I

SURFACE AREAS OF DIFFERENT SIZES OF WHEAT KERNELS

| SAMPLE AND KERNEL SIZE | AVERAGE WEIGHT PER KERNEL | AVERAGE AREA PER KERNEL | AREA PER GRAM |
|---|---|---|---|
| | *g* | *sq mm* | *sq mm* |
| Sample A: Size 1 | 0.0153 | 25 | 1612 |
| 2 | .0213 | 28 | 1350 |
| 3 | .0273 | 33 | 1199 |
| 4 | .0377 | 44 | 1173 |
| 5 | .0481 | 51 | 1015 |
| Sample B: Size 1 | .0120 | 18 | 1520 |
| 2 | .0207 | 27 | 1308 |
| 3 | .0293 | 35 | 1157 |
| 4 | .0380 | 43 | 1124 |
| 5 | .0425 | 45 | 1023 |
| Sample C: Size 1 | .0121 | 22 | 1834 |
| 2 | .0204 | 27 | 1322 |
| 3 | .0277 | 33 | 1183 |
| 4 | 0.0357 | 38 | 1065 |

ide solution changes continuously during the reaction period: The greater the surface to be measured, the greater the change in rate. The curve of Fig. 1, therefore, probably should not be a straight line, but for the present use a straight line is satisfactory.

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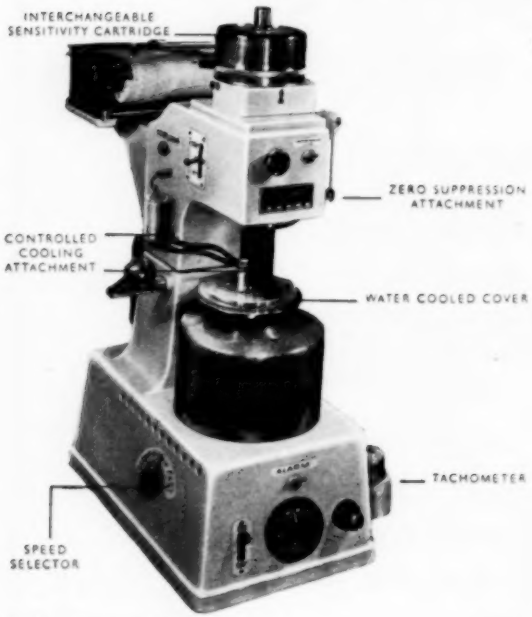


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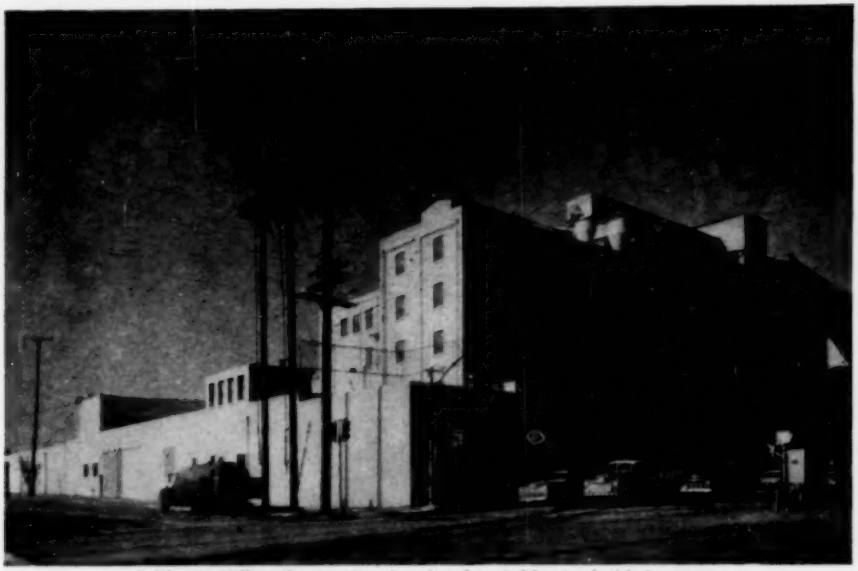

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